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Original Article

Chemical Characterization and Biological Evaluation of Two Alkyl-Substituted Phenyl Aldimines

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ABSTRACT

Background of study: Schiff bases are organic compounds noted for their versatility and exhibition of diverse biological activities including antioxidant, antibacterial, antifungal and antiviral amongst others. Hence, their significance in medicinal chemistry.
Objectives: The worrisome resistance of microbes to anti-infective drugs in clinical therapy prompted the search for pharmaceutically active compounds with proven activities for treating disease conditions. Schiff base synthesis involving benzaldehyde and two alkyl amines was considered.
Methodology: Benzaldehyde was separately reacted with hexyl amine and heptyl amine leading to imines. The antioxidant activity of the compounds was evaluated using the DPPH test. A comparison of the antioxidant activities so obtained was done to determine if the synthesized imines would show better activities than benzaldehyde and Vitamin C. The agar diffusion method was used for screening the compounds against Staphylococcus aureus, Escherichia coli, Candida albicans and Aspergillus niger for both antibacterial and antifungal potentials respectively.
Results: Physico-chemical determinations and IR spectral technique have revealed the nomenclatures of both imines to be N-hexyl-1-phenyl methanimine and N-heptyl-1-phenyl methanimine respectively. The two imines did not give any antioxidant activity. However, the antibacterial and antifungal activities elicited by the imines showed concentration-dependency with Schiff base 2 being more antimicrobial than Schiff base 1.
Conclusion: The results obtained especially from the antimicrobial tests show the two aldimines could be promising lead templates in the search for more efficacious biological agents especially in synergistic antimicrobial co-administration and formulation studies in drug development.

Introduction

The history of Schiff bases or imines (named after the German chemist Hugo Schiff), can be traced back to the mid-19th century (1864), They are a class of organic compounds with a carbon-nitrogen double bond (-HC=N) which is formed in the condensation reactions between primary/aromatic amines and carbonyl compounds (aldehydes / ketones) leading to the elimination of water [1]. Imines have gained attention not only for their synthetic utility and versatility but also for their diverse biological activities. These include but not limited to antimicrobial, anti-cancer, anti-inflammatory, anti-malarial, enzyme inhibition, chelation and anagelsic activities amongst many others [2,3]. The search for new and effective pharmacological

and pharmaceutical agents is an ongoing effort in medicinal chemistry research. The rising incidence of resistance to current clinical antibiotics, antibacterial and antifungal drugs and toxicity issues concerning antioxidant drugs thereby posing critical problems to public health safety worldwide has prompted the exploration of alternative compounds [4]. Hence, the choice of Schiff synthesis involving benzaldehyde whose IUPAC name is benzene carbaldehyde. This aromatic aldehyde has an almond color and occurs naturally in the kernels of stone fruits (apricots, cherries and peaches), almond oil and cassia oil. Also, it serves as a synthetic intermediate in the manufacture of ephedrine, pseudoephedrine and methamphetamine [5], cinnamic acid, benzoin and various aromatic pharmaceutical dyes. Also, it is used as a flavoring agent in food baking industry and fragrance in skin care cosmetic perfumery. Furthermore, it has found applications in manufacturing of dyes, soaps, odor control and as a preservative due to its antimicrobial properties [6]. The high reactivity of this aromatic aldehyde facilitates the formation of imines which are stable even at relatively mild reaction conditions [7]. Furthermore, the hydroxyl substituted analogues of this aldehyde afford imines with highly remarkable activities [8]. Furthermore, vanillin (a derivative of benzaldehyde) with electron donating groups found in it has demonstrated antioxidant, antibacterial and antifungal activities in Schiff bases obtained thereby [3,9]. Despite these developments, relatively few studies have explored the syntheses and biological profiling of Schiff bases functionalized with long-chain aliphatic amines with benzaldevdye. These amines could confer enhanced lipophilicity and membrane permeability on the resulting imines and possibly obtaining improved targeted biological activities. Consequently, this study was conceived with the aim of synthesizing imines using benzaldehyde and two aliphatic amines (hexyl-amine and heptyl-amine). In addition, the aldimines so obtained would be screened for antioxidant, antibacterial and antifungal activities with a view to rationally evaluating if they could become templates for drug development and formulation studies.

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Materials and Methods

Reagents/chemicals

DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) was obtained from Tianjin Kernel Chemical Reagent Company, China while ciprofloxacin, fluconazole and Vitamin C tablets were sourced from Gemini Pharmaceuticals, Nigeria. Reagents namely, acetic acid (glacial), acetone, benzaldehyde, ethanol, hexyl-amine, heptyl-amine, methanol and toluene were purchased as AnaLAR Grade Chemicals from Sigma Aldrich Chemicals, Germany.

Miscibility of benzaldehyde

Benzaldehyde (1 mL) was added to separate test tubes containing 3 mL each of acetone, ethanol, distilled water, methanol and toulene separately and observation for miscibility was recorded.

Solubility tests of synthesized imines (Schiff base 1 and Schiff base 2)

Each imine (0.03 g) was added to 3 mL each of the following solvents namely, acetone, ethanol, distilled water, methanol and toulene separately and observation was made for complete dissolution (solubility) or otherwise.

Determination of melting point

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

Synthesis of Schiff base 1

The Schiff base was synthesized using the method described by Sharma and Diwan, with slight modifications. Benzaldehyde (10 mL) was measured and transferred into a conical flask containing ethanol (50 m L). Hexyl-amine (10 m L) was added to the solution in the flask. A few drops of glacial acetic acid were added drop wise and the resulting deep yellow coloured solution heated under reflux at 100°C for 5 h. Cool water (10 m L) was added to the solution resulting in the formation of a deep yellow oily phase. The reaction vessel was placed on ice for twenty minutes and then the content of the reaction vessel transferred into a separating funnel. The oily phase was collected and transferred into a petri-dish and kept at room temperature overnight. The amorphous yellow precipitate formed was collected and weighed [10].

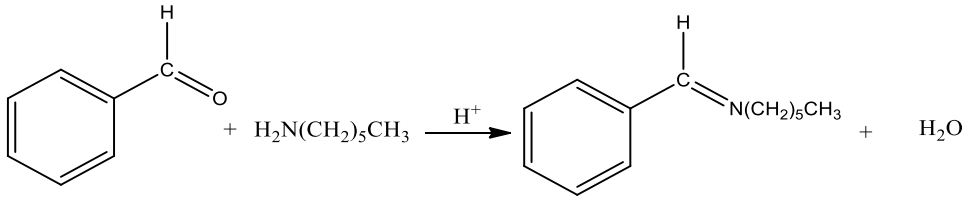

Figure 1: Synthesis of Schiff base 1 (N-hexyl-1-phenyl methanimine (N-hexyl benzene methanimine).

Synthesis of Schiff base 2

The Schiff base was synthesized using the method described by Sharma and Diwan, with slight modifications. Benzaldehyde (10 mL) was measured and transferred into a conical flask containing ethanol (50 m L). Heptyl-amine (10 m L) was added to the solution in the flask. A few drops of glacial acetic acid were added drop wise, and the resulting deep yellow coloured solution heated under reflux at 100°C for 5 h. Cool water (10 m L) was added to the solution resulting in the formation of a light yellow oily phase. The reaction vessel was placed on ice for some minutes and then the reaction mixture transferred into a separating funnel. The oily phase was collected and transferred into a petri-dish and kept at room temperature overnight. The white precipitate formed was re-crystallized using ethanol (10 mL) to give a white crystalline solid. The product was then collected and weighed [10].

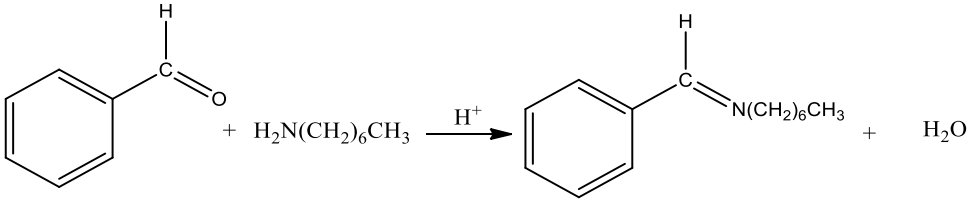

Figure 2: Synthesis of Schiff base 2 (N-heptyl-1-phenyl methanimine (N-heptyl benzene methanimine).

Determination of optical rotation and refractive indices of benzaldehyde and the synthesized imines

These experiments were performed using a polarimeter (ADP-220, Bellingham Stanley, England) and a refractometer (WAY-15, Abbe, England) respectively with slight modifications [3]. Each sample (0.04 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 taken at the wavelength (λ) of sodium D line (589.3nm) at 20.5ºC. Similarly, the refractive index of sample was obtained on the refractometer at the wavelength (λ) of sodium D line (589.3 nm) at 20.5ºC [11].

Thin-layer chromatography of benzaldehyde and synthesized imines

Benzaldehyde (0.03 g) and imines (0.03 g) were separately dissolved in methanol (2 mL) and a little portion of each spotted 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 achieved.

The retardation factor (R_F) was then computed thus:

$$R_F = \frac{\text{distance moved by spot}}{\text{distance moved by solvent front}}$$

Infra-red spectroscopy (FTIR) of synthesized imines

The imines (0.02 g) each was analyzed for IR characteristics using the FTIR 84005 Spectrophotometer (Shimadzu, Japan).

Ultra-violet/visible spectroscopy of benzaldehyde and synthesized imines

Benzaldehyde (0.01 g) and imines (0.01 g) were analyzed for UV/VS absorption characteristics using the Jenway 6405 UV/VS Spectrophotometer.

Determination of antioxidant activity

Spectrophotometric evaluation 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 [12]. This reaction is routinely monitored by absorption spectrophotometry.

Preparation of calibration curve for DPPH reagent

This experiment was carried out as described [13,14] 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_{max} 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.

Determination of the antioxidant activity of benzaldehyde, synthesized imines 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 ± 2^0C), 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_{max} 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. Benzaldehyde /synthesized imine / Vitamin C concentration providing 50 % inhibition (IC_{50}) was calculated from a graph of inhibition percentage against the concentration of the benzaldehyde / synthesized imine /Vitamin C. Vitamin C was used as a standard antioxidant drug [15].

Antimicrobial Tests

The micro-organisms used in this study, namely, *Staphylococcus aureus* (NCTC 4534), *Escherichia coli* (NCTC 1068). *Candida albicans* (NCYC 2439) and *Apergillus niger* (NCYC 2764) were previously isolated from specimens of diarrheal stool, abscesses, patients' urinary samples, wounds and vaginal swabs obtained from the Medical Laboratory, University of Uyo Health Centre, Uyo. The isolates collected in sterile bottles were identified and typed by convectional biochemical tests and then refrigerated at -5^0C at the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy prior to use [16]. 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 labelled 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 5.00 mm. The wells were equidistant from each other and the edge of the plate [17]. Concentrations of 20 mg mL^{-1} of benzaldehyde, 10 mg mL^{-1} and 20 mg mL^{-1} of aldimines were introduced into the wells. Also, different concentrations of 5 µg mL^{-1} ciprofloxacin and 1mg mL^{-1} of fluconazole both (Fidson Chemicals Limited, Lagos, Nigeria) and 50 % methanol were introduced into separate wells as positive and negative controls respectively [18]. 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± 2^0C for 24 h. The zones of inhibition were afterwards then measured in millimeters (mm).

Results

Table1: Preparation of calibration curve For DPPH at λ_{max} 517 nm.

Concentration (%w/v)	Average absorbance
0.0004	0.156
0.0008	0.304
0.0012	0.445
0.0016	0.596
0.0024	0.851
0.0036	1.204

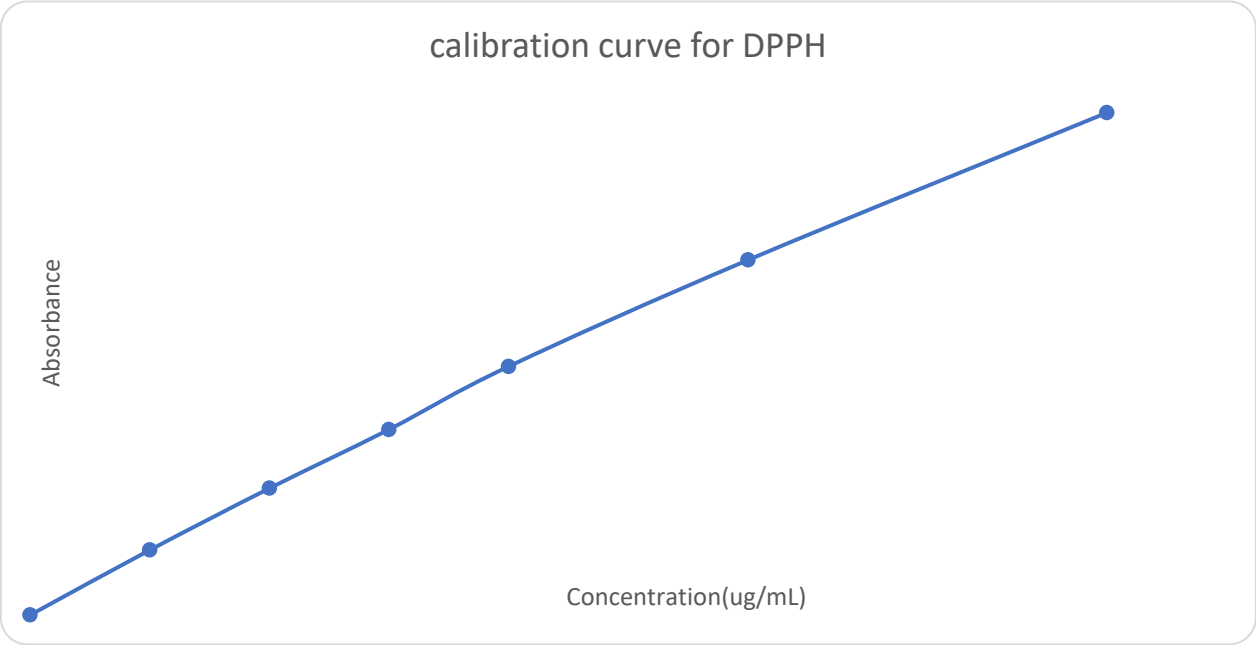

Figure 3: Graph of calibration curve for DPPH.

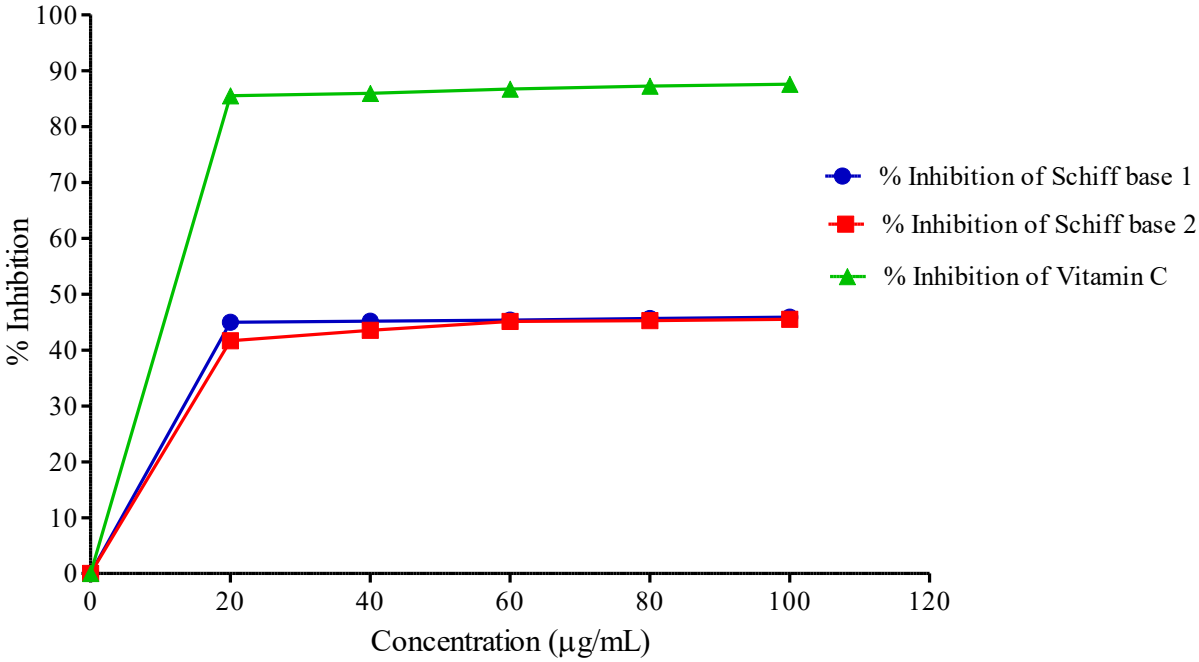

Figure 4: Percentage inhibition (%) *vs* Concentration curves of benzaldehyde, Schiff base 1, Schiff base 2 and Vitamin C.

Table 2: Absorbance of Schiff base 1, Schiff base 2, benzaldehyde and Vitamin C incubated with DPPH at different concentrations (mg mL^{-1}) at λ_{max} 517 nm (Blank absorbance of 0.004 % w/v DPPH reagent: 0.025) (± 0.004).

Concentration	Schiff base 1	Schiff base 2	Benzaldehyde	Vitamin C
0	0	0	0	0
20	0.726	0.769	0.722	0.116
40	0.723	0.745	0.720	0.113
60	0.721	0.724	0.716	0.110
80	0.717	0.722	0.710	0.100
100	0.714	0.719	0.705	0.098

Schiff base 1= *(N-hexyl-1-phenyl methanimine (N-hexyl benzene methanimine)*
Schiff base 2 *= (N-heptyl-1-phenyl methanimine (N-heptyl benzene methanimine)*
DPPH *= 2, 2-Diphenyl-1-picryhydrazyl hydrate*

Table 3: Radical scavenging activity (percentage inhibition %) of samples at different concentrations (mg mL^{-1}) and IC_{50} of samples (± 0.02).

Concentration(mg/mL)	Schiff base 1	Schiff base 2	Benzaldehyde	Vitamin C
0	0	0	0	0
20	44.998	41.658	42.194	85.494
40	45.185	43.517	45.264	85.934
60	45.337	45.109	45.403	86.703
80	45.643	45.261	45.552	87.252
100	45.868	45.489	45.587	87.582

Table 4: Radical scavenging activity (Antioxidant activity IC_{50} (µgmL^{-1}) of Schiff base 1, Schiff base 2, benzaldehyde and Vitamin C.

Sample	IC_{50} (µgmL^{-1})
Schiff base 1	NG
Schiff base 2	NG
Benzaldehyde	NG
Vitamin C	0.12

RSA *% (PI %) = Radical Scavenging Activity (Percentage Inhibition %)*
IC_{50} *= Concentration at which 50 % of DPPH is scavenged or inhibited*
NG = *Not Regressed*

Table 5: Antibacterial tests of benzaldehyde, Schiff base 1 and Schiff base 2 at different concentrations on test microbes in 50 % methanol (± 0.01 mm).

Microbe	Benzaldehyde 20 mg L^{-1}	Schiff base 1 10 mg mL^{-1}	Schiff base 1 20 mg L^{-1}	Schiff base 2 10 mg mL^{-1}	Schiff base 2 20 mg mL^{-1}	Ciprofloxacin 5 µg mL^{-1}	MeOH/ H_2O (1:1)
S. aureus (NCTC 4534)	17.87	20.92	23.35	25.02	30.52	45.69	5.00
E. coli (NCTC 1068)	13.90	15.82	18.09	20.82	25.01	40.09	5.00

The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +5.00) mm* ***NCTC* *- National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9, UK.*

Table 6: Antifungal screening of benzaldehyde, Schiff base 1 and Schiff base 2 at different concentrations on test microbes in 50 % methanol (± 0.01 mm).

Microbe	Benzaldehyde 20 mg L^{-1}	Schiff base 1 10 mg mL^{-1}	Schiff base 1 20 mg L^{-1}	Schiff base 2 10 mg mL^{-1}	Schiff base 2 20 mg mL^{-1}	Fluconazole 1 mg mL^{-1}	MeOH/ H_2O (1:1)
C. albicans (NCYC 2439)	5.00	27.80	37.28	29.92	40.40	25.91	5.00
A. niger (NCYC 2764)	5.00	28.45	38.76	25.84	37.43	14.89	5.00

The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +5.00) mm; **NCYC- National Collection of Yeast Cultures, UK.*

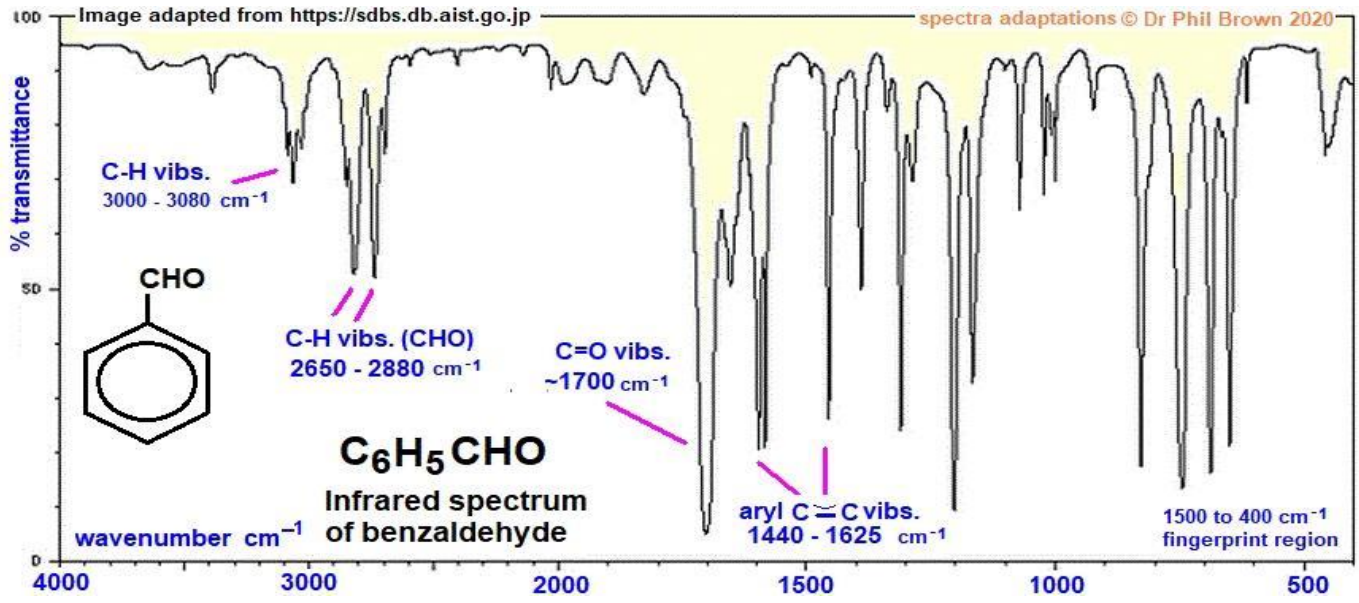

Figure 5: Library specimen of Infra-red spectrum of benzaldehyde.

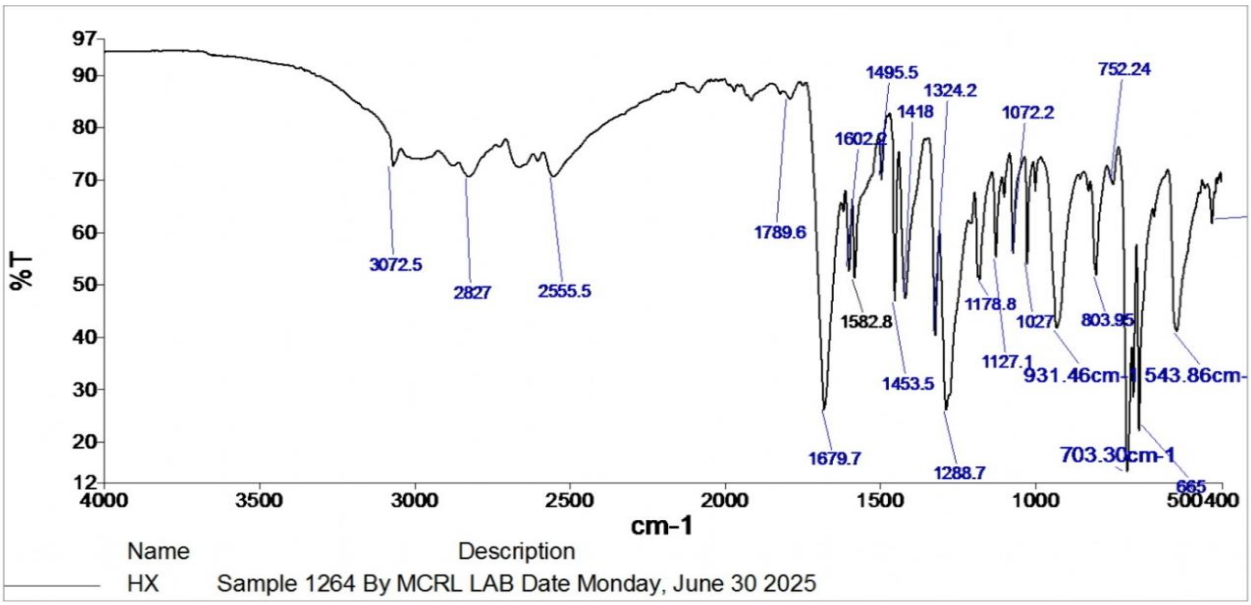

Figure 6: Infra-red spectrum of Schiff base 1.

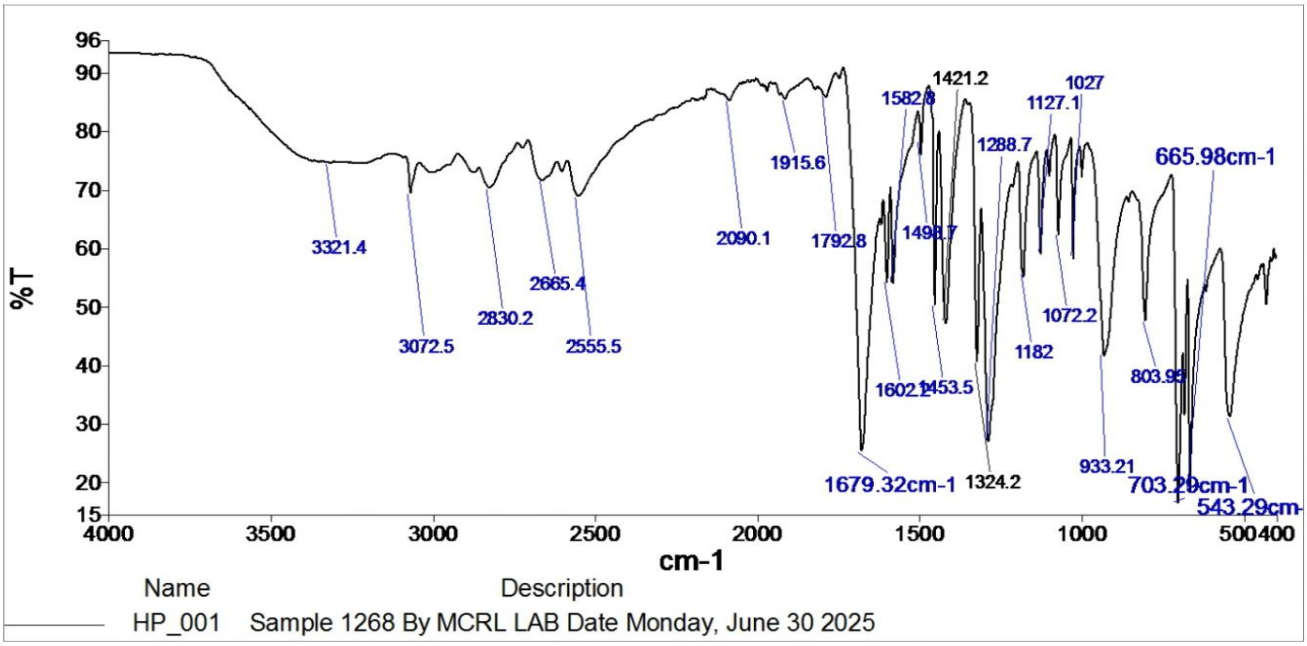

Figure 7: Infra-red spectrum of Schiff base 2.

Benzaldehyde,: C_7H_6O; mol. wt. (106.17 g/mol); pale yellow liquid; $[n]_D^{20}$ (1.5450); $[\alpha]_D^{20}$ (0); λ_{max} (248 nm); R_F (0.74); FTIR (cm^{-1}):1625 (-Ar-C=C), 1700 (-C=O of -CHO), 2880 (-CH vibration in -CHO) and 3080 (-CH vibration bending mode on -Ar ring).

Schiff base 1 (N-hexyl-1-phenyl methanimine (N-hexyl benzene methanimine): $C_{13}H_{19}N$; mol. wt. (190.02 g/mol); amorphous yellow compound; m.pt. (166-168 ^{0}C); $[n]_D^{20}$ (1.5953); $[\alpha]_D^{20}$ (0), λ_{max} (261nm); R_F (0.79); FTIR (cm^{-1}): 703 and 823 (finger print region, alkyl bending modes), 1582 (Ar-C=C), 1679 (-HC=N, azomethine), 2827 and 3072 (-CH bending mode on the -Ar ring).

Schiff base 2 (N-heptyl-1-phenyl methanimine (N-heptyl benzene methanimine): $C_{14}H_{21}N$; mol. wt. (204.06 g/mol); white crystalline compound; m.pt. (Not available); $[n]_D^{20}$ (1.6059); $[\alpha]_D^{20}$ (0); λ_{max} (262 nm); R_F (0.83); FTIR (cm^{-1}): 703, 823 and 833 (finger print region, alkyl bending modes), 1602 (-Ar-C=C), 1679 (-HC=N, azomethine), 2930 and 3072 (-CH bending modes on the -Ar ring).

Discussion

Chemical characterization

Monographic evaluations of substances/reagents used in chemical reactions (synthesis/ derivatizations/assays) are of utmost necessity for the purposes of establishing their identity, purity, chemical stability, molecular integrity and suitability for use prior to any study. Consequently, benzaldehyde, an almond colored aromatic aldehyde with a flavor and fragrance was subjected to these determinations. This aldehyde was observed to be miscible with acetone, ethanol, methanol and toluene but not water. Furthermore, the determined optical rotation and refractive index values are consistent with those in literature. The observed UV absorption characteristics of benzaldehyde at λ_{max} (248 nm) indicate the presence of electron clouds delocalized over -Ar-C=C and -CH=O chromophores respectively. Its retardation factor R_F (0.74) connotes that it is nonpolar and expectedly weakly retarded on the silica plate. In addition, the different peaks in IR spectrum of the amine as highlighted in **Figure 4** are consistent with those obtained in literature. The Schiff base condensation reaction between benzaldehyde and hexyl amine gave an amorphous yellow imine (**Schiff base 1)** whose IUPAC nomenclature has been identified to be **N-hexyl-1-phenyl methanimine (N-hexyl benzene methanimine)** by a coupling of physico-chemical values and the FTIR spectral technique. It has a faint fragrance. It is an imine which belongs to the class of compounds known as ketimines/aldimines. These compounds are obtained by the *in-situ* removal of water through condensation between amines and carbonyl group containing compounds such as ketones or aldehydes in the presence of an acid or base and under heat. An aldimine results if an aldehyde is used while a ketimine is obtained when ketone is otherwise used. The UV absorption profile at λ_{max} (261 nm) of this aldimine which is higher than that shown by benzaldehyde

conveys information about the presence of electrons clouds over -Ar-C=C in addition to the -HC=N (imine, azomethine). Its retardation factor R_F (0.79) is indicative of this base being comparably more non-polar than the aldehyde because of the incorporated hexyl group $(-CH_2)_5CH_3$ making it more lipophillic and more poorly retarded on a chemically hydrophobic silica gel plate. The IR spectral matrix of **Schiff base 1** shows absorption peaks 703 and 823, 1582, 1679, 2827 and 3072 cm^{-1} which are characteristically diagnostic of alkyl bending modes in the fingerprint region, -Ar-C=C, -HC=N (imine, azomethine) and -CH (bending mode on -Ar ring) respectively. The reaction between benzaldehyde and heptyl amine afforded needle-like white crystalline imine (**Schiff base 2).** Similarly, a combination of the above mentioned techniques and procedures was deployed in its identification. Consequently, the IUPAC nomenclature has been identified to be **N-heptyl-1-phenyl methanimine (N-heptyl benzene methanimine**. Its UV absorption profile at λ_{max} (262 nm) is slightly higher than that of Schiff base 1 at 261 and benzaldehyde at 240 nm respectively. Hence, the electron-cloud densities are localized over -Ar-C=C and -HC=N (imine, azomethine) as have also been pin-pointed in the **Schiff base 1** above. In addition, the retardation factor R_F (0.83) is indicative of relatively more highlighted non-polar nature of heptyl group $(-CH_2)_6CH_3$ group evidently making this imine much more lipophillic than both benzaldehyde and **Schiff base 1** at 0.74 and 0.79 respectively. Consequently, it was observed to be much more poorly retarded on the silica plate. The IR spectral matrix of **Schiff base 2** shows evident absorption strectchings at 703, 823, 833, 1602, 1679, 2930 and 3072 cm^{-1} which are routinely characteristic of alkyl bending modes, -Ar-C=C, -HC=N (azomethine), and -CH (bending mode on -Ar ring). respectively. A strikingly noticeable observation in the IR matrices of both imines (aldimines) is the disappearance of the -HC=O peak at 1700 cm^{-1} and replacement with that of the azomethine at 1679 cm^{-1} expressly showing that both condensation reactions were successful**.** In addition, both imines were very soluble in all the organic solvents used and insoluble in water. Physico-chemical parameters are somewhat important in identifying compounds. In the light of this refractive index and optical rotation are used in the qualitative and quantitative evaluations of substances. These parameters are used to confirm the purity, identity and integrity of substances. They are both ideally measured at the wavelength (λ) of Na-D light (589.3 nm) and a temperature of 20.5 ^{0}C. It is worth noting that the refractive index of a substance is an indication of the number, type of atoms and chemical groups in the substance. Each atom or group in the substance contributes its individual and distinct refractivity which adds eventually to the total refractivity of a substance. Hence, the refractive indices of the compounds were then determined. Benzaldehyde, Schiff base 1 and Schiff base 2 gave refractive indices of 1.5450, 1.5953 and 1.6059 respectively. In addition, all the three compounds demonstrated optical rotation $[\alpha]_D^{20}$ of 0 ^{implying} that none had chiral centres and consequently optically inactive. Furthermore, none of these compounds would show 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) [19].

Biological evaluations
Antioxidant assay

Absorption spectrophotometry remains the standard test in evaluating antioxidant potential of compounds such as chemicals/ drugs/ plant isolates/ synthesized products [20]. The preparation of a calibration curve is of immense necessity before the reagent can be used in such a bench-top assay. Hence, the primary standard, DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) reagent was put through this experiment with the aim of ascertaining its purity and suitability prior to the antioxidant determinations. The Beer-Lambert's Law is adopted for such determinations [21]. A calibration curve was obtained which obeyed the Law as can been seen in **Figure 3** showing a straight line which passed through the origin. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_{max} 517 nm. Ideally a color change from purple to yellow is expected to indicate a conversion (reduction) of DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to the non-radical form; 1, 1-diphenyl-2-picryl hydrazine [22]. Substances which are capable of donating electrons or hydrogen atoms can effectively do this. The absorbance of DPPH was observed to decrease as the concentration of added free-radical scavenger (benzaldehyde,/Schiff base/Vitamin C) increased which suggested that the DPPH reagent was being reduced as **Table 2** attests. Furthermore, **Table 3** displays the radical scavenging activity (RSA %) or percentage inhibition (PI %) and the computed IC_{50} values of benzaldehyde /Schiff base/Vitamin C. The RSA % is a measure of the antioxidant activity of benzaldehyde /Schiff base/Vitamin C. Interestingly, it was observed that benzaldehyde and the Schiff bases did not convert the DPPH's purple color to yellow as expected but merely bleached it to faint purple. Vanillin, a

nutraceutical with a benzaldehyde moiety has electron-donating groups such as methoxy and hydroxyl at meta and para positions which must have impacted on the color change witnessed (purple to yellow) and hence the documented antioxidant activities recorded in previous studies [3,9]. The antioxidant activity (IC_{50}) of benzaldehyde, Schiff base 1 and Schiff base 2 could not be regressed (**NG**) from the PI % Vs Concentration Curves as displayed in **Figure 4** most probably because of the absence of any electron-donating groups such as found in vanillin which could have donated electrons or hydrogen atoms in the antioxidant assays. Hence, it is safe to infer that the two imines and benzaldehyde are not antioxidant. Vitamin C, being a standard antioxidant drug expectedly exhibited a significant IC_{50} of 0.12 μg mL^{-1}.

Antibacterial profiling

The microbes used in this study reflected a broad spectrum *viz;* one (1) gram (+) bacterium namely, *S. aureus* and one (1) gram (-) bacterial species *E. coli*. The two Schiff bases (1 and 2) both recorded concentration-dependency activity against the test organisms at 10 and 20 mg L^{-1} respectively. Both imines were also more bacteriostatic against the two bacteria than benzaldehyde as can be seen in **Table 5.** Schiff base 2 was evidently more suppressive of both *S. aureus* and *E. coli* than Schiff base 1. This is indeed note-worthy because the longer the alkyl chain attached, the more lipophillic the imine becomes hereby possessing a much-enhanced penetrating ability across the membranes of the bacteria. Previously documented reports have shown that Schiff bases are antibacterial [3,9]. It is probably safe to infer from these observations that the two imines are promising molecular drug templates in the quest for more efficacious antibacterial compounds, especially for synergistic activity when co-administered in the face of chronically prevalent microbial resistance witnessed currently in clinical bactericidal chemotherapy.

Antifungal evaluation

The antifungal screenings were done with *C. albacans* and *A. niger*. Similarly, the recorded activities by the two imines were also concentration-dependent just as seen in the antibacterial tests. Furthermore, Schiff base 2 was more anti-fungal than Schiff base 1 as displayed in **Table 6.** Both also gave better activities than benzaldehyde. Similarly, the length of the attached heptyl group enhanced the diffusion of the imine into the fungal membranes just as was witnessed in the antibacterial tests. Previous studies on some cinnamyl Schiff bases have shown remarkable anti-fungal activity against both *C. albicans* and *Aspergillus species* [23]. Hence, the results obtained from this present research were not unexpected. However, more studies on synergistic co-administration for antifungal potential of these two aldimnes are recommended going forward.

Conclusion

This study obtained two aldimines namely, N-hexyl-1-phenyl methanimine (N-hexyl benzene methanimine) **(Schiff base 1)** and N-heptyl-1-phenyl methanimine (N-heptyl benzene methanimine) **(Schiff base 2)** respectively. Both Schiff bases did not demonstrate antioxidant activity. However, the two imines elicited dose-dependent antibacterial activity at concentrations used and were both significantly suppressive of tested bacteria. **Schiff base 2** was particularly more active than **Schiff base 1, most** likely on account of the longer heptyl chain facilitating better diffusion of chemical materials into the bacterial membrane. It should be indicated that both imines were more bacteriostatic against *S. aureus* than *E. coli* as expected. Similarly, both compounds also demonstrated antifungal potential against C. *albicans* and *A. niger* showing dose-dependency. Furthermore, both compounds were less suppressive of *A. niger.* Similarly, Schiff base 2 was also more antifungal than Schiff base 1. Further research is recommended especially in synergistic antibacterial and antifungal co-administration studies with a view to possibly ameliorating the prevalence of microbial resistance to antibiotics and antifungal drugs already in therapy.

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Conflict of Interest

None declared.

Author Contributions

All the authors contributed to the study.

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