Synthesis, infra red characterization and antimicrobial evaluation of Schiff bases derived from 1, 3-diphenylprop-2-en-1-one and 1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one
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ABSTRACT
Chalcones were synthesized via Claisen-Schmidt condensation between various substituted acetophenone and benzaldehyde using 20% cold alcoholic KOH as catalyst. Two Schiff bases were synthesized through the condensation reaction of the synthesized chalcones with 2,4-dinitrophenylhydrazine using concentrated H₂SO₄ as catalyst. The antimicrobial properties of the Schiff bases were determined using the standard disc diffusion method of anti-microbial sensitivity according to CLSI. The results of anti-microbial analysis were compared to ciprofloxacin and ampicillin as reference standards. The first Schiff base was N-(2,4-Dinitro-phenyl)-N’-(1,3-diphenyl-allylidene)-hydrazine (SB₁), a hydrazone of 1,3-diphenylprop-2-en-1-one and the second was N-[3-(4-chloro-phenyl)-1-phenyl-allylidene]-N’-(2,4-dinitro-phenyl)-hydrazine (SB₂), a hydrazone of 1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one. SB₁ had a percentage yield of 97.57% and a decomposition temperature of 231-234°C while SB₂ gave 98.74% as percentage yield and a melting point of 216-218°C. SB₁ showed anti-microbial properties while SB₂ showed no anti-microbial properties when both were tested against Staphylococcus aureus and Pseudomonas aeruginosa at a concentration of 10 mg/mL. SB₁ had a diameter zone of inhibition of 4-6 mm for P. aeruginosa and 7-9 mm for S. aureus. SB₁ was more effective against g positive than g negative bacteria at a concentration of 10 mg/L. The presence of the major absorption band in the range of 350-375 nm, attributable to the n-π transition of aromatic ketones in the UV spectra of the synthesized chalcones gave credence to their proposed aromatic structure. The presence of strong bands at 1604 cm⁻¹ for SB₁ and 1601 cm⁻¹ for SB₂ and the absence of carbonyl oxygen absorption band in their IR spectra gave credence to the formation of Schiff bases.
Key words: Antimicrobial, Schiff base, ligand, chalcones, inhibition, synthesis

INTRODUCTION
The susceptibility of mankind as well as plants and animals to pathogens necessitates the development and introduction of substances with biological activities, whether synthetic or natural. There are various synthetic compounds with biological activities in chemistry [1]. Synthetic drugs are designed to mimic the actions of organic botanical compounds, but they often contain highly processed chemicals. However, the body has difficulty recognizing synthetic drugs, which makes them harder to metabolize. For this reason, these drugs are more likely to induce toxicity [2]. Modern synthetic chemistry broke the sole dependence of human wellbeing on plant-derived medicines, making western medications fundamentally subject to pharmaceuticals dependent on manufactured or inferred molecules applied orally, topically or infused [3]. However, there are some synthetic drugs which are believed to provide relief from ailments, inhibit or suppress pathogen growth and possess low toxicity levels. One of such substances are a group of compounds referred to as Schiff bases. Schiff bases are the condensation products of primary amines with carbonyl compounds [4]. These compounds are commonly known as anils, imines or azomethines. They are referred to as imines when the nitrogen atom is attached to a hydrogen or to an organic group. They are called anils when the nitrogen atom is attached to a phenyl group which may or may not be substituted. They can also be referred to as hydrazones if the primary amine is a hydrazine. Primary amines and carbonyl groups react to give Schiff bases according to the following scheme:
Shiff bases can be distinguished from other chemical compounds by their common structural feature which is the azomethine group having a general representation: RHC=NR’, where R and R’ can be alkyl, aryl, cyclo alkyl or heterocyclic group which may be variously substituted.

Studies have shown that the presence of a lone pair of electrons in the sp^2 hybridized orbital of the nitrogen atom of the azomethine group is of considerable chemical and biological importance [5].

Shiff bases have excellent chelating property. They are generally bidentate, tridentate or polydentate ligands capable of forming very stable complexes with transition metals. Their coordinating ability is enhanced if they bear a functional group, such as –OH or –SH, close enough to the site of condensation in such a way that a five- or six-membered ring can be formed when reacting with a metal ion [6].

Shiff bases resulting from aromatic aldehydes ortho-substituted with a hydroxyl group are of interest to researchers because of their ability to act as bidentate ligands for transition metal ions [7]. Schiff bases derived from salicyaldehydes show anti-cancer activity [8] as well as plant growth regulatory [5], antimicrobial and antimitotic activities [9]. A large number of different Schiff base ligands have been used as cation carriers in potentiometric as a result of their excellent selectivity, sensitivity and stability for specific metal ions such as Ag^{2+}, Al^{3+}, Co^{2+}, Cu^{2+}, Hg^{2+} and many other transition metals [10]. Certain cobalt Schiff base complexes are potential antiviral agents [11].

Shiff bases have also been studied because of their catalytic properties. For example, they show catalytic activity in the hydrogenation of olefins [12].

**SYNTHESIS OF CHALCONES**

Shiff bases are active against a wide range of organisms such as *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus polymxna*, *Trychophyton gypseum*, *Mycobacteria*, *Erysiphe grisea* and *Plasmopora viticola* [13].

Shiff bases have been used as corrosion inhibitors, as a result of their ability to spontaneously form a monolayer on the surface to be protected [14]. The major interaction between the surface of the metal and the corrosion inhibitor is chemisorptions [15]. The biological, analytical and industrial usefulness of complexes of Schiff bases make their further investigations desirable as a result of their preparative accessibility, structural variability and electronic properties allowing ancillary modifications based on systematic reactivity studies to be carried out.

Over several decades, it has been recorded that pathogens have evolved, becoming resistant to anti-biotics [16]. This poses serious threats to antibiotic drug therapy. The problems of modern synthetic drugs necessitate the creation of cheaper drug substances with less hazards and lower toxicity as well activities against known-drug resistant micro-organisms [17]. It is hoped that synthesized Schiff bases meet these criteria.

The replacement of C=O of chalcones with C=N of Schiff base increases the anti-microbial properties of the compound [18].

The purpose of this study therefore is to synthesize, characterize some Schiff bases and evaluate their anti-microbial properties against known drug resistant micro-organisms.

**MATERIALS AND METHODS**

\[
\begin{align*}
\text{R}= & \text{R'} & \text{R}= & \text{R'} & \text{R}= & \text{R'} \\
\text{substituted} & \text{Acetophenone} & \text{substituted} & \text{Benzaldehyde} & \text{Ethanol}/20\%\text{KOH} & \text{ice bath}/<10^\circ\text{C}, 2\text{ h} \\
\rightarrow & \text{Chalcone} & \text{(R and R' = H or substituent group)}
\end{align*}
\]
Scheme 2: Representative reaction for the synthesis of the chalcones

**Synthesis of 1,3-Diphenylprop-2-en-1-one (CA₁)**

To 30 mL of absolute ethanol, 2 mL (0.017 moles) of acetophenone (99%, 1.03 g/mL) and 1.73 mL (0.017 moles) of benzaldehyde (98.5%, 1.04 g/mL) were added in a 100 mL conical flask mounted on a magnetic stirrer and stirred till the mixture became homogeneous. The mixture was then immersed in a bigger container holding crushed ice and placed back on the stirrer. Stirring was continued while keeping the temperature below 10°C and 5 mL of cold KOH (20%) was added gradually. Stirring was again continued for another 2 hours, during which TLC was used to monitor the progress of the reaction until the starting materials disappeared. The reaction mixture was then covered and kept in the refrigerator for 24 hours.

**SYNTHESIS OF THE SCHIFF BASES**

**Scheme 3:** Condensation of a chalcone with a hydrazine

**Synthesis of N-(2,4-Dinitro-phenyl)-N'-{(1,3-diphenyl-allylidene)-hydrazine (SB₁)}**

Crystals of 1,3-diphenylprop-2-en-1-one (0.50 g) were weighed and dissolved in 20 mL of methanol in a 100 mL conical flask. Its molar equivalent of 2,4-dinitrophenylhydrazone (0.48 g) was also weighed and mixed with 20 mL of methanol in a 100 mL conical flask. The mixtures in the two conical flasks were stirred and boiled separately to a temperature of 50-60°C. 0.80 mL of concentrated H₂SO₄ was added to the suspension of 2,4-dinitrophenylhydrazone in methanol and it was poured into the heated solution of 1,3-diphenylprop-2-en-1-one in methanol. The resulting mixture was stirred and boiled at 50-60°C for 5 minutes and allowed to cool to 25°C.

The reaction mixture was then filtered and air-dried to obtain a yield of 0.91 g. The progress of the reaction was monitored using TLC. The melting point was determined by using the open capillary method and was uncorrected.

**Synthesis of 1-Phenyl-3-(4-chlorophenyl)-prop-2-en-1-one (CA₂)**

To 30 mL of absolute ethanol, 2 mL (0.017 moles) of acetophenone (99%, 1.03 g/mL) and its molar equivalent of 4-chlorobenzaldehyde (2.39 g) were weighed separately and mixed together in a 100 mL. The reaction was carried out according to the procedure described in (I) (a) above to obtain a yield of 3.10 g.

Thereafter, the mixture was acidified with 5 mL of 50% glacial acetic acid until it was neutral to litmus paper. It was then filtered, residue washed with distilled water and dried to obtain a yield of 3.31 g. Its purity was ascertained using TLC and the melting point was also determined by using the open capillary method and was uncorrected.
added to the suspension of 2,4-dinitrophenylhydrazine in methanol and it was poured into the heated solution of 1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one in methanol. The resulting mixture was stirred and boiled at 50-60°C for 5 minutes and allowed to cool to 25°C. The reaction mixture was then filtered and air-dried to obtain a yield of 0.86 g. The progress of the reaction was monitored using TLC. The melting point was determined by using the open capillary method and was uncorrected.

ANTI-MICROBIAL SENSITIVITY TEST
Micro organisms
Two micro-organisms, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology, University of Benin, Benin City. Pure amoxicillin and ciprofloxacin from Oxoide™, used as reference standard for comparing results were obtained from the University of Benin Teaching Hospital.

Preparation of Materials
Glassware such as petri dishes, test tubes, glass rod, pipette, measuring cylinders, medicine bottles and beakers were washed and dried in the oven. They were then wrapped in aluminum foil and sterilized in an autoclave. The nutrient agar was cooled and put into sterilized petri dishes which were then covered.

0.05g of the synthesized Schiff bases were mixed with 5 mL of distilled water in transparent medicine bottles, and sterilized sensitivity discs were put into the bottles. The bottles were made to stand for 30 minutes at room temperature for diffusion to take place.

Inoculation
Muller-Hinton agar medium was used for this experiment. Disc diffusion method was used in the anti-microbial sensitivity screening.

The petri dishes containing agar were inoculated with the laboratory isolates of the microorganisms, then an ‘L-shaped’ rod was used to spread the micro-organisms on the agar in the petri dishes. The sensitivity discs were then carefully placed on the agar and the petri dishes were immediately covered and incubated at 37 ± 1°C for about 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the paper discs was measured. The experiment was carried out in triplicate for each of the samples.

RESULTS AND DISCUSSION
Chalcones
Spectroscopic evaluation

*CA₁*: 1,3-diphenylprop-2-en-1-one
IR (KBr, cm⁻¹), 1774 (C=O), 1558 (CH=CH), 360 (C-H stretch), UV (λ \text{max} 350-375 nm (n-π)), 280-310 nm (aromatic rings). Yield= 95.36%
CA₂:1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one  
IR (KBr, cm⁻¹), 1652 (C=O), 1337 (CH=CH), 327 (C-H stretch), UV (λ max 360-375 nm (n-π*), 280-310 nm (aromatic rings). Yield= 74.61%

Figure 1: CA₂: Infrared spectrum for 1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one

The UV spectra of the synthesized chalcones all show the major absorption band, λ max at within the range of 350-375 nm which may be attributed to the n-π* transitions of the whole molecule. Bands in the range of 280-310 nm, exclusive to transitions of aromatic rings indicate the presence of aromatic rings.

Table 1: Properties of the synthesized chalcones

| Chalcone | Colour     | Physical state         | Melting point | *Retention factor (Rf) |
|----------|------------|------------------------|---------------|------------------------|
| CA₁      | Milky      | Crystalline powder     | 42-44°C       | 0.65                   |
| CA₂      | Milky      | Non-crystalline lumps of solid | 55-60°C       | 0.70                   |

* n-hexane/ethylacetate 5:1  
CA₁ = 1,3-diphenylprop-2-en-1-one; CA₂ = 1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one

Table 1 shows the physical properties of the synthesized chalcones. The high retention factors of the synthesized chalcones show they are quite non-polar while the melting points of the compounds indicate that they are relatively pure compounds.

Table 2: Solubility of synthesized chalcones in various solvents

| Chalcone | n-hexane  | Ethylacetate          | Chloroform    | Acetone         | Acetic acid    |
|----------|-----------|-----------------------|---------------|-----------------|----------------|
| CA₁      | Insoluble | Completely soluble     | Completely    | Completely      | Insoluble      |
|          |           |                       | soluble       |                 |                |
| CA₂      | Insoluble | Completely soluble     | Completely    | Completely      | Insoluble      |
|          |           |                       | soluble       |                 |                |

* CA₁ = 1,3-diphenylprop-2-en-1-one; CA₂ = 1-phenyl-3-(4-chlorophenyl)-prop-2-en-1-one
Table 2 above shows the solubility profile of the chalcones. The solubility of the synthesized chalcones in acetone and ethyl acetate which are moderately polar solvents and their insolubility in n-hexane show that the chalcones are mildly polar.

Schiff base

Spectroscopic evaluation

SB₁: N-(2,4-Dinitro-phenyl)-N’-(1,3-diphenyl-allylidene)-hydrazine
IR (KBr, cm⁻¹), 1604 (C=N), 3437 and 3257 shoulder (N-H stretch), 372 (C-H stretch), Yield= 97.56%

SB₂: N-[3-(4-Chloro-phenyl)-1-phenyl-allylidene]-N’-(2,4-dinitro-phenyl)-hydrazine
IR (KBr, cm⁻¹), 1601 (C=N), 3440 and 3274 shoulder (N-H stretch), 704(C-Cl stretch), Yield= 98.74%
Table 3: Properties of the synthesized Schiff bases (2,4-dinitrophenylhydrazones)

| Schiff base | Colour  | Physical state | Melting point |
|-------------|---------|----------------|---------------|
| SB₁         | Orange  | Powder         | 231-234 °C    |
| SB₂         | Red     | Powder         | 216-218 °C    |

The range of the melting point of the synthesized Schiff bases show that they are relatively pure.

Table 4: Solubility of the synthesized Schiff bases in various solvents

| Schiff base | n-hexane  | Ethyl acetate | Chloroform | Acetone | Glacial acetic acid |
|-------------|-----------|---------------|------------|---------|--------------------|
| SB₁         | Insoluble | Completely soluble | Completely soluble | Insoluble |                   |
| SB₂         | Insoluble | Completely soluble | Completely soluble | Insoluble |                   |

SB₁ = N-(2,4-Dinitro-phenyl)-N’-(1,3-diphenyl-allylidene)-hydrazine

SB₂ = N-[3-(4-Chloro-phenyl)-1-phenyl-allylidene]-N’-(2,4-dinitro-phenyl)-hydrazine

The insolubility of the synthesized Schiff bases in non-polar n-hexane as well as their solubility in acetone and ethyl acetate which are polar shows that the Schiff bases have some degree of polarity.

Table 5: Anti-microbial Evaluation

| Isolates               | Zone of Inhibition | Antimicrobial susceptibility |
|------------------------|--------------------|------------------------------|
|                        | SB₁                | SB₂                          |
| *Pseudomonas aeruginosa* | 4 - 6 mm          | 0 mm                         |
|                        | sensitive          | resistant                    |
| *Staphylococcus aureus* | 7 - 9 mm          | 0 mm                         |
|                        | sensitive          | resistant                    |

Concentration of shiff bases SB₁ and SB₂ = 10mg/mL. diameter zone of inhibition + 0.5 mm

SB₁ = N-(2,4-Dinitro-phenyl)-N’-(1,3-diphenyl-allylidene)-hydrazine

SB₂ = N-[3-(4-Chloro-phenyl)-1-phenyl-allylidene]-N’-(2,4-dinitro-phenyl)-hydrazine

The results of anti-microbial analysis using amoxicillin and ciprofloxacin as reference standards are displayed in Table 5 above. It is interesting to note that Schiff base SB₁ showed zones of inhibition for both *P. aeruginosa* and *S. aureus*. However, the Schiff base SB₂ showed no zone of inhibition for *P. aeruginosa* and *S. aureus*.

CONCLUSION

Schiff bases were successfully synthesized via the condensation of chalcones with 2,4-dinitrophenylhydrazine. The physico-chemical properties of the synthesized compounds may be as a result of the various substituents attached to their moieties. Anti-microbial evaluations carried out show that the Schiff base: N-(2,4-Dinitro-phenyl)-N’-(1,3-diphenyl-allylidene)-hydrazine (SB₁) showed biological activity against *S. aureus* and *P. aeruginosa* at a concentration of 10 mg/mL while the second Schiff base: N-[3-(4-Chloro-phenyl)-1-phenyl-allylidene]-N’-(2,4-dinitro-phenyl)-hydrazine (SB₂) showed no biological activity against *S. aureus* and *P. aeruginosa* at the same concentration of 10 mg/mL.

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