Original Research Article

Microwave-assisted Synthesis, Characterization, Antimicrobial and Antioxidant Activities of 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one and its Co(II) Complex

Abstract

Aims: To develop a simple, efficient microwave-assisted synthetic method to preparing 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one and its Co(II) complex, characterize and ascertain their biological significance.

Place and Duration of Study: School of Chemistry, Cardiff University, Wales, United Kingdom (November, 2007); Department of Chemistry & Industrial Chemistry, Department of Biological Sciences and Department of Biochemistry, Bowen University, Iwo, Nigeria (September 2015 and June 2018).

Methodology: *N*-benzylisatin and 4-methylaniline were microwave irradiated in acetic acid to give 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one ($C_{22}H_{18}N_2O$, **L;** 58 %). **L** was microwave irradiated with $CoCl_2.6H_2O$ in ethanol to yield its Co(II) complex. **L** was characterized using ^{1}H-NMR, ^{13}C-NMR, IR and Electronic spectra analyses, exact mass and melting point. IR and Electronic Spectra analyses with melting point confirmed the Co(II) complex was formed. The *in-vitro* antimicrobial activities of **L** were evaluated against three gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and Haemolytic *Staphylococcus aureus*), three gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella* sp.) and three fungi (*Aspergillus niger*, *Trichoderma viride* and *Penicillium citrinum*). The IC_{50} of **L** and its Co(II) complex were carried out against DPPH, H_2O_2 and NO radicals, as well as their reducing power abilities.

Results: Antimicrobial studies revealed **L** was active against *Pseudomonas aeruginosa* with a high zone of inhibition (thrice that of tetracycline) and *Penicillium citrinum*. The IC_{50} of **L** against DPPH, H_2O_2 and NO radicals were 0.561 ± 0.02, 0.3 ± 0.01 and 0.53 ± 0.01 µg/ml respectively, while they were 0.200 ± 0.01, 0.9 ± 0.02 and 0.26± 0.03 µg/ml for Co(II) complex. Their reducing power abilities at IC_{50} were 0.53 µg/ml (for **L**) and 0.6 ± 0.03 µg/ml (the complex).

Conclusion: L was synthesized within 15 min and its Co(II) complex within 5 min. L showed good free radical scavenging activities and reducing power when compared with ascorbic acid, while its Co(II) complex even performed better.

Key Words:

Microwave-assisted, synthesis, 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one, Antimicrobial, Antioxidant, Activities

1.0 Introduction

The use of microwave irradiation synthetic method has recently gained much attention. It speedily facilitates the synthesis of new chemical entities with pharmaceutical applications by improving the efficiency of such chemical reactions [1, 2]. Isatin is a versatile precursor in fine organic/inorganic syntheses of many amine-, ether-, nitrile- and oxazole- derivatives [3, 4, 5], as well as several metal complexes [6, 7, 8, 9], with antimicrobial [10], antiviral, anti-inflammatory, analgesic [7, 11] and anticonvulsant activities [12]. Quite an innumerable number of isatin derivatives have proven to be good therapeutic agents for several coronary diseases including ischemic heart disease, cardiac arryhythmia, hypertension, depression and even cancer [6]. The preparation and x-ray cryastallographic structure of *N*-benzylindole-2, 3-dione (*N*-benzylisatin) has been reported [13]. *N*-alkylated isatins have also been reported to possess interesting chemistry and pharmacological activities such as antibacterial, antiviral

and anticancer [14, 15]. Therefore the need to synthesize biologically active Schiff bases of *N*-benzyl isatin and their complexes using a simple and time efficient method and also establishing their biological significance are the propelling forces for this research. We have reported the single crystal structure of 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one grown after conventional refluxing of isatin and P-toluidine for 6 hours [16]. We hereby report the convenient microwave-assisted synthesis of 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one (**L**) within 15 minutes, 30 seconds, its full spectroscopic characterization, antimicrobial studies, free radical scavenging activities and the reducing power assay. This report also includes the successful microwave irradiation synthesis (within 4 minutes, 55 seconds), free radical scavenging activities and the reducing power assay of its Co(II) metal complex.

2.0 Experimental Details

2.1 Chemical

Isatin, P-Toluidine, $CoCl_2.6H_2O$, 1,1-Diphenyl-2 picryl hydrazyl (DPPH), ascorbic acid, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium chloride, sodium nitroprusside and sulphanilamide were obtained from Aldrich. All solvents used (ethanol, methanol, chloroform, acetone, dichloromethane, pyridine, diethyl ether, n-hexane, and *N*,*N*-dimethylformamide; DMF) were purchased as analytical grades from Sigma-Aldrich and SAARChem.

2.2 Instrumentation

Microwave experiments were performed inside a domestic oven (24 L, 800W light-up Dial Microwave). ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on Bruker 400 MHz Spectrometer. The infrared spectra were recorded on Agilent Cary 630 FTIR spectrometer. The UV-Visible spectra were recorded on a Shimadzu UV-1800 spectrometer. Mass spectrum was determined using a Fisons VG Quattro Spectrometer. The purity of the compounds were checked by Thin-Layer Chromatography (TLC) carried out on Silica Gel 60 F254 alumina plates (E Merk) using appropriate solvent mixtures of diethyl ether: petroleum ether or chloroform as the eluent and visualized in UV chamber (365 nm). Melting points were determined using a Gallen kemp variable heater apparatus.

2.3.0 Synthetic Work

2.3.1 Preparation of 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one (L)

This was done in two steps.

2.3.2 Synthesis of *N*-benzylisatin ($C_{15}H_{11}NO_2$)

N-benzylisatin was prepared according to literature [13, 16].

2.3.3 Microwave Synthesis of 1-benzyl-3-[(4methylphenyl)imino]indolin-2-one ($C_{22}H_{18}N_2O$; L)

N-Benzylisatin (1 g; 4.22 mmol) and P-toluidine (0.45 g; 4.22 mmol) were both dissolved in 20 ml acetic acid by applying heat. The mixture was poured inside a beaker and put inside a 24 L, 800W light-up Dial Microwave. This was first irradiated for 7 minutes, 30 seconds (medium) and thereafter 8 minutes (high) at 30 seconds intervals. A brown oil was got. This was recrystalized in ethanol and left to cool. A light orange solid 0.8 g (58%) was obtained, m.p. 152.4-154.6 ^{o}C (154 ^{o}C from Ikotun *et al.* 2012b); λ_{max} (CH_3OH)/nm 417, 300, 250 ($E/dm^3mol^{-1}cm^{-1}$ 3139, 4559, and 24914); υ_{max}/cm^{-1} 1716 (C=O), 1597 and 1683 (C=N + C=C), 1467 (CH_{bend}), 1100 and 1079 (C-N + C-C); δH (400 MHz, DMSO-d_6)/ppm 5.00 (2H, s, CH_2), 6.55-7.70 (13H, m, ArH); δC (DMSO-d_6)/ppm 162.90 (C=O), 154.23 (C=N), 148.18 (Cq), 147.30 (Cq), 136.38 (Cq), 134.86 (Cq), 134.60 (CH), 130.5 (CH), 129.25 (CH), 129.20 (CH), 129.15 (CH), 128.03 (CH), 127.84 (CH), 125.54 (CH), 122.87 (CH), 120.44 (CH), 117.95 (CH), 115.90 (CH), 111.10 (CH), 110.00 (CH), 21.06 (**C**H_3), 43.36 (CH_2); $^{m}/_{z}$ (ESI) 327 ($M+1^+$ peak, 100 %).

2.3.4 Syntheses of the Co(II) Complex

L (1 g; 0.00422 mol) was dissolved in 20 ml ethanol. $CoCl_2.6H_2O$ (1 g; 0.00422 mol) was added to the solution. This was microwave irradiated (medium) for 4 minutes, 55 seconds at an interval of 30 seconds to yield a brown amorphous solid, 0.64 g (20 %), m.p. › 320 ^oC; λ_{max} (CH_3OH)/nm 666, 619, 423, 310, 288 (15015, 16151, 23640, 32258 and 34722 cm^{-1}); υ_{max}/cm^{-1} 1698 (C=O), 1590 and 1614 (C=N + C=C), 1461 (CH_{bend}) and 1098 (C-N + C-C).

2.4.0 Antimicrobial Activity

The synthesized compounds were screened for *in-vitro* antibacterial and antifungal activities using Mueller-Hinton agar (MHA) and Potato dextrose agar (PDA) media. The antibacterial activity was evaluated against three gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and Haemolytic *Staphylococcus aureus*) and three gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* sp.). Also, the antifungal activities of the compounds were evaluated against three fungi (*Aspergillus niger*, *Trichoderma viride* and *Penicillium citrinum*). Preliminary identification of the bacteria was carried out following the methods described by [17]. Tetracycline (30 µg; antibiotic test kit) was used as a standard drug for the bacteria, while dimethylformamide (DMF) was used as control.

2.4.1 Antibacterial Test

An 18 h culture of each test bacteria was suspended in a sterile universal bottle containing nutrient broth. Normal saline was added gradually in order to compare its turbidity to that of 0.5 McFarland standard corresponding to approximately 10^8 cells/ml. This was diluted to produce 10^6 cells/ml used for the experiment [18]. For the antibacterial susceptibility test, one milliliter (1 mL) of test organism (10^6 cells/mL) was inoculated into Petri plates (90 mm diameter). 19 ml molten Mueller Hinton agar (MHA) sterilized at 121 ^oC for 15 min was also added. The plates were shaken gently for even mixing. The agar was left on the bench to solidify. Disc diffusion method was used to evaluate the antimicrobial activities of compounds. Filter paper discs were cut (diameter 8 mm) and the discs sterilized. **L** was dissolved in dimethylformamide (DMF) at a concentration of 100 µg/mL. The discs were impregnated with the solution (100 µg/mL), picked with sterile forceps and placed on the MHA plates containing the test organisms [19]. The plates were left on the bench for 1 h to diffuse before incubating at 37 ^oC for 24 h.

2.4.2 Antifungal Test

The fungal isolates were allowed to grow on Potato dextrose agar (PDA) (LabM) at 25 °C for 5 – 7 days to sporulate. After sporulation, the fungal spores were harvested by pouring a mixture of sterile glycerol and distilled water unto the surface of the plate. The spores were scraped using a sterile glass rod. The harvested fungal spores were standardized to 10^6 spores per ml. One milliliter of the standardized spore suspension was evenly spread on solidified PDA (LabM) plates using a glass spreader. The plates were placed on the work bench for 1h for the spore suspension to diffuse into the agar. The sterile discs were impregnated with the test compounds and placed aseptically using sterile forceps on the surface of the agar plates. The plates were then allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the compounds into the media. Plates were incubated at 25 °C for 96 h and observed for zones of inhibition. Activity was evaluated by measuring the diameters of zones of growth inhibition in triplicates and the mean of three results determined.

2.4.3 Minimum Inhibitory Concentration (MIC)

This was carried out by adding 10, 5.0, 2.5, 1.25, 0.625, and 0.3125 µg/ml of **L** into test tubes containing sterile nutrient broth. *Pseudomonas aeruginosa* was thereafter introduced into the broths containing different concentrations of **L**. The tubes were then incubated for 24 h at 37

^{o}C. The MIC was taken as the lowest concentration of **L** that did not permit any visible growth [20].

2.5.0 Antioxidant Properties

The antioxidant properties determined were the free radical scavenging activities and the reducing power abilities of the synthesized compounds. These values were determined as a mean ± standard deviation of three different readings.

2.5.1 Free radical scavenging activity and reducing power assays

All synthesized compounds were screened for *in vitro* free radical scavenging activities using DPPH, H_2O_2, nitric oxide (NO) radical scavenging activity assay, and reducing power assay.

2.5.2 DPPH radical scavenging activity

DPPH radical scavenging activity of the compounds was determined using the method of Blios (1958) [21]**.** To 0.1 ml of different concentrations (0.1 to 1.0 µg/ml) of the test compounds, 2.5 ml of methanol and 0.5 ml of 0.2 mM DPPH solutions were added and mixed thoroughly and the absorbance was read at 517 nm against blank. Ascorbic acid was used as a reference standard. The IC_{50} (Inhibitory concentration of the test compound required to scavenge 50 % of DPPH free radicals) was thereafter calculated.

2.5.3 H_2O_2 radical scavenging activity

This was determined according to literature [22, 23]. The solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (pH 7.4). Various concentrations (0.1 to 1.0 µg/ml) of 1 ml of test compounds and standard were added to 2 ml of H_2O_2. Absorbance of H_2O_2 at 230 nm was determined 10 min later against a blank solution containing the phosphate buffered saline without H_2O_2. The IC_{50} of H_2O_2 was thereafter calculated.

2.5.4 NO radical scavenging activity

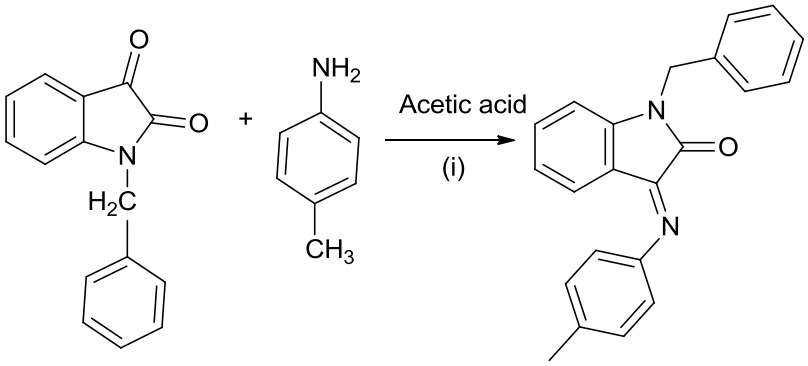
Nitric Oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by Griess reaction [24, 25]. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline and test compounds in different concentrations was incubated at 25 ^{0}C for 150 min. At intervals, samples (0.5 ml) of incubation solution were removed and 0.5 ml of Griess reagent (1 % sulphanilamide, 2 % H_3PO_4 and 0.1 % naphthylethylene diamine dihydrochloride) was added. The absorbance of the chromophore formed was measured at 546 nm.

2.5.5 Reducing power

The reducing power of the test compounds was carried out according to literature [21] (Blios 1958). 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and potassium ferric cyanide (1 % w/v) were added to 1.0 mL of the sample dissolved in DMF. The resulting mixture was incubated at 50 ^{0}C for 20 mins, followed by the addition of 2.5 mL trichloroacetic acid (10 % w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL) mixed with distilled water (2.5 mL) and ferric chloride (0.1 % w/v), the absorbance was then measured at 700 nm against blank sample.

3.0 RESULTS AND DISCUSSION

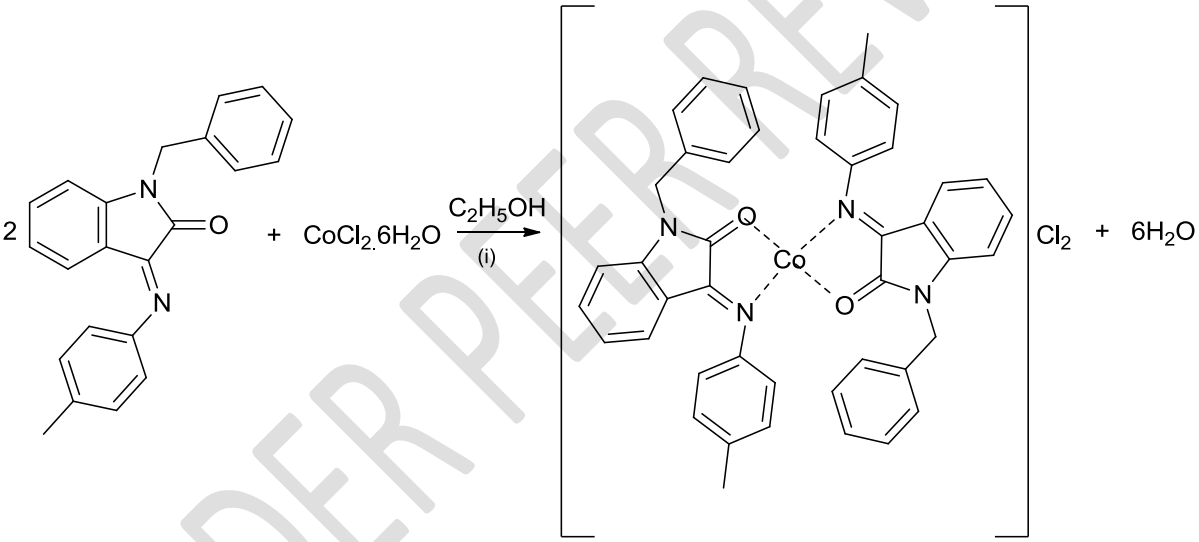
The reaction for the microwave synthesis of **L** from *N*-benzylisatin is presented as Scheme 1.



(i) Microwave Irradiation; 7 min, 30 sec (Medium) and 8 min (High)

Scheme 1: Microwave synthesis of 1-benzyl-3-[(4-methylphenyl)imino]-indolin-2-one ($C_{22}H_{18}N_2O$; **L**) from the prepared *N*-benzylisatin

Scheme 2 presents the microwave synthesis of the Cobalt(II) complex similar to Ikotun *et al.* (2012a) [10]. This could also possibly explain the low yield, since the metal has picked up two molecules of the ligand making the reaction ratio to be 1:2 (Metal : Ligand).



(i) Microwave Irradiation; 4 min,55 sec (Medium)

Scheme 2: Microwave Synthesis of the Co(II) complex

3.1 Infrared spectra of L and its Co(II) Complex

The characteristic vibrational frequencies in the infrared spectra have been identified by comparing the spectrum of the Schiff base with the Co(II) complex. These have been presented in Table 1.

Table 1: Relevant Infrared Spectral Data of the L and its Co(II) Complex

COMPOUND	υ(C=O) (cm^{-1})	υ(C=N+C=C) (cm^{-1})	υ(CH)bend (cm^{-1})	υ(C-N+C-C) (cm^{-1})
L ($C_{22}H_{18}N_2O$)	1716 s	1683 s 1597 m	1467 s	1100 m 1079 m

Co(II) Complex	1698 s	1614 s 1590 m	1461 s	- 1098 m

Note: υ stretching; m, medium and s, strong

The assignments of these absorption bands have also been made by comparing the spectra of the compounds with reported literature on transition metal complexes of isatin Schiff bases [6, 10]. There are two potential donor sites in **L**. These are the isatin nitrogen and the isatin oxygen. The FTIR spectrum of **L** ($C_{22}H_{18}N_2O$) showed a strong intensity band at 1716 cm^{-1} attributed to υ(C=O) stretching vibration. This band has undergone a shift to a lower frequency of 1698 cm^{-1} in the spectrum of its Co(II) complex signifying the involvement of the keto oxygen in coordination to Co(II). The spectrum of **L** showed a strong and a medium band at 1683 and 1597 cm^{-1} attributed to υ(C=N + C=C) stretching vibration. These bands have moved to lower frequencies of 1614 and 1590 cm^{-1}. This also signifies the involvement of the imine nitrogen in coordination to Co(II). The strong band appearing at 1467 cm^{-1} in the spectrum of **L** was attributed to the υ(CH_{bend}) vibration and it has shifted slightly to a lower frequency of 1461 cm^{-1} in the spectrum of its complex. The medium bands appearing at 1100 and 1079 cm^{-1} in the spectrum of **L** were attributed to υ(C-N + C-C) stretching vibration. One of these bands has disappeared, while the other appeared at 1098 cm^{-1} in the spectrum of the Co(II) complex. All these confirm the formation of the Co(II) complex of this ligand.

3.2 ^{1}H- and ^{13}C-NMR Spectra of L ($C_{22}H_{18}N_2O$)

In the ^{1}H-NMR spectrum of **L**, a singlet at δ 5.00 ppm appeared, which is due to the methylene group. The aromatic protons appeared as groups of multiplets in the range 6.55-7.70 ppm. In the ^{13}C-NMR spectrum (DMSO-d_6) of this compound, the expected 22 signals were observed as follows (δ ppm): 162.90 (C=O), 154.23 (C=N), 148.18 (Cq), 147.30 (Cq), 136.38 (Cq), 134.86 (Cq), 134.60 (CH), 130.5 (CH), 129.25 (CH), 129.20 (CH), 129.15 (CH), 128.03 (CH), 127.84 (CH), 125.54 (CH), 122.87 (CH), 120.44 (CH), 117.95 (CH), 115.90 (CH), 111.10 (CH), 110.00 (CH), 21.06 (**C**H_3). The peak that appeared at 43.36 ppm is characteristic of the **C**H_2 group.

3.3 Mass Spectrum of L ($C_{22}H_{18}N_2O$)

Exact mass analysis of L showed the elemental composition to be $C_{22}H_{18}N_2O$, which corresponds to the expected molecular formula. It revealed the molecular ion peak at $^{m}/_{z}$ (ESI) 327 (100 %) corresponding to $[M+H]^{+}$.

3.4 UV-Visible

The ultraviolet spectra analyses of the prepared compounds have been presented as Table 2.

Table 2: Electronic Spectra of L and its Co(II) Complex

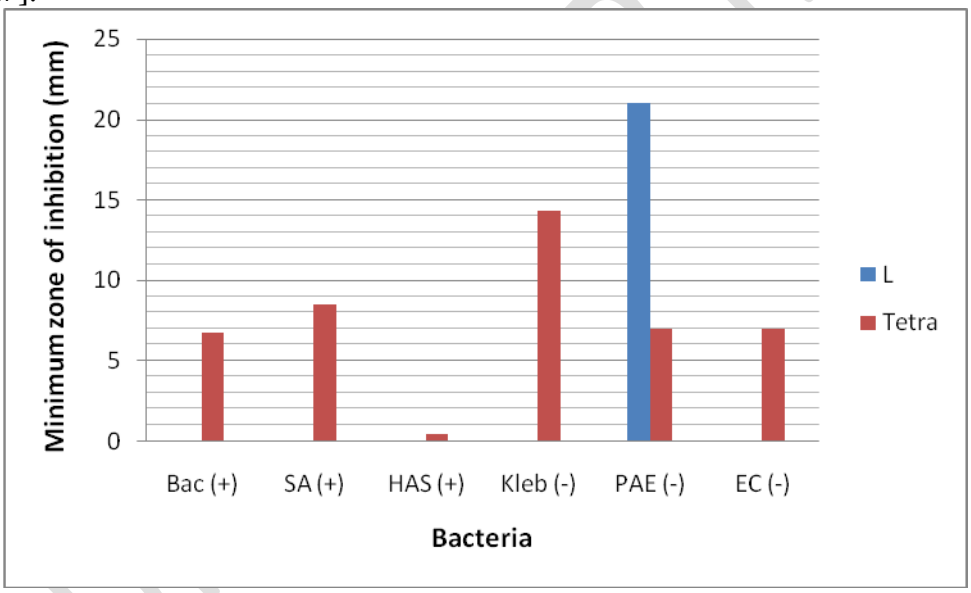
Compound	Band position (nm)	Band position (cm^{-1})	Band Assignment
L	417	23,981	n –π*
	300	33,333	π –π*
	250	40,000	π –π*
Co(II) Complex	666	15,015	d–d
	619	16,151	d–d
	423	23,640	n –π*

	310	32,258	π –π*
	228	34,722	π –π*

The absorption bands have been assigned by comparing the spectra of the compounds with reported literature on transition metal complexes of isatin Schiff bases [6, 10]. The spectrum of **L** showed absorption bands at 33,333 and 40,000 cm^{-1} (300 and 250 nm) which have been assigned to π – π* transition. The band appearing at 23,981 cm^{-1} (417 nm) has been assigned to n - π* transition. The Co(II) complex was characterized with bands appearing at 15,015 and 16,151 cm^{-1} (666 and 619 nm), which have been assigned as d-d transitions. The Co(II) complex spectrum also showed a band at 23,640 cm^{-1} (423 nm) assigned to n –π* transition, as well as bands appearing at 32,258 and 34,722 cm^{-1} (310 and 288 nm) assigned to π –π* transitions.

3.5 Antimicrobial Activities

Figure 1 presents the comparison of the results of the antibacterial studies of **L** and tetracycline as a bar chat. This shows it was only active against *Pseudomonas aeruginosa*, which is a very drug resistant bacterium that causes wound infections including gangrin [26, 27].



Note Keys:

Bac = *Bacillus subtillis* (+)	Kleb (-) = *Klebsiella sp.*
SA = *Staphyllocus aureus* (+)	PAE (-) = *Pseudomonas aeroginosa*
HAS = Haemolytic *Staphyllocus aureus* (+)	EC (-) = *Escherichia coli*
L = $C_{22}H_{18}N_2O$ (**L**)	Tetra = Tetracycline

Figure 1: Comparative Results of the Antibacterial Activities of **L** and Tetracycline

L had a high zone of inhibition against this bacterium, which was thrice that of tetracycline. Thus it can be developed as a drug against *Pseudomonas aeruginosa*. Figure 2 also presents the results of the antifungal studies of **L** as a bar chat.

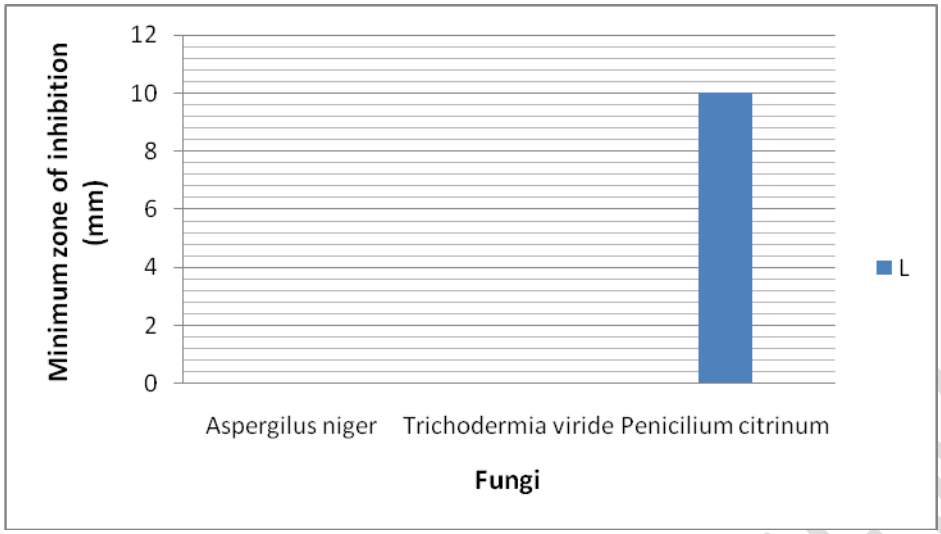


Figure 2: Results of the Antifungal Studies of **L**

It revealed that **L** was active against one of the tested fungi (*Penicillium citrinum*) and could be a potential drug against this fungus *Penicillium citrinum.*

3.6.0 Antioxidant Activities

The results of the antioxidant activities are presented below.

3.6.1 DPPH Radical Scavenging Activity

Figure 3 presents the comparison of the results of DPPH radical scavenging activities of **L**, its Co(II) complex and ascorbic acid as a bar chart.

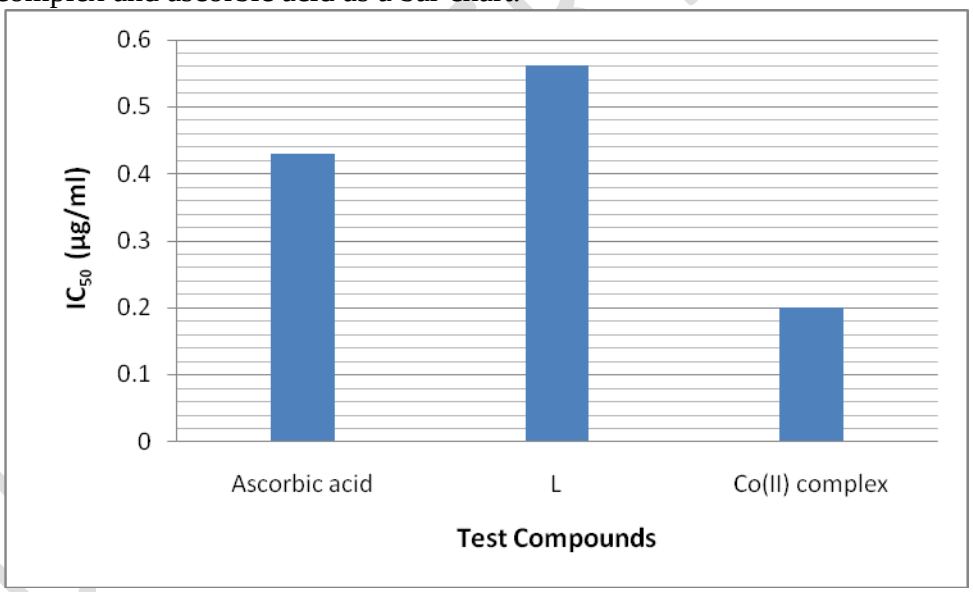


Figure 3: IC_{50} values of test compounds for DPPH radical scavenging activity

DPPH mimics many free radicals produced in the biological system which have a stable but highly delocalized spare electron. They are not readily broken down to less reactive species. They either form dimmers or attack macromolecules in the biological system which leads to abrogated cellular function, carcinogenesis, aging or cell death. However, they become less reactive when an antioxidant donates hydrogen atom to their molecules or a metal complex molecule chelates them. The Cobalt(II) complex had a lower IC_{50} value compared to **L** and ascorbic acid. This shows the potency of this metal complex in scavenging a charged and highly reactive radical like DPPH in a biological system. It is most likely that the positively charged cobalt ion in this metal complex molecule readily chelates the nitrogen donor (DPPH

molecule) to prevent its attacks on macromolecules in a biological system. Therefore, the Co(II) metal complex possesses better DPPH radical scavenging activity than its ligand (**L**) and ascorbic acid.

3.6.2 H_2O_2 radical scavenging activity

Figure 4 presents the comparison of the results of H_2O_2 radical scavenging activity for **L**, its Co(II) complex and ascorbic acid as a bar chart.

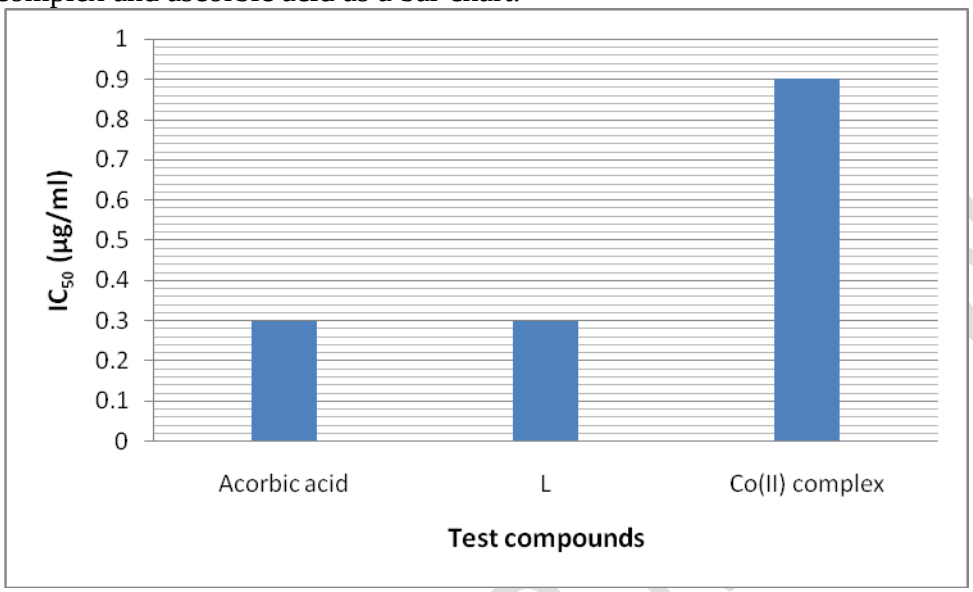


Figure 4: IC_{50} values of test compounds for H_2O_2 radical scavenging activity

Hydrogen peroxide is a very reactive and unstable radical which the human body produces during infection. It rapidly decomposes into a more reactive but relatively stable hydroxyl radical ($OH^{\cdot}$). Both of them combat the invading microorganisms and eventually kill it. They initiate lipid peroxidation, DNA mutation and oxidative stress in the microorganism which eventually lead to their death. Although the human body has its mechanism of detoxifying these radicals, their sustained production after the death of the microorganisms could lead to similar effects in the human body. **L** showed higher potency in scavenging hydrogen peroxide than its Co(II) complex. This means that **L** is highly sensitive to hydrogen peroxide at a very low concentration. The higher hydrogen peroxide scavenging activity of **L** can be explained by the presence of the carbonyl and imino groups in its molecule, which show high sensitivity to H_2O_2. However, the metal complex has both the oxygen of the carbonyl group and the imine nitrogen involved in coordination to the Co(II) ion. This is a possible reason for its reduced ability in scavenging hydrogen peroxide. And this could invariably account for its bactericidal effect by sustaining the hydrogen peroxide oxidation of macromolecules in the bacterial cell which could lead to the death, inhibition of growth or reduced population of the bacterial cell.

3.6.3 NO radical scavenging activity

Figure 5 presents comparison of the results of NO radical scavenging activities for **L**, its Co(II) complex and ascorbic acid as a bar chart.

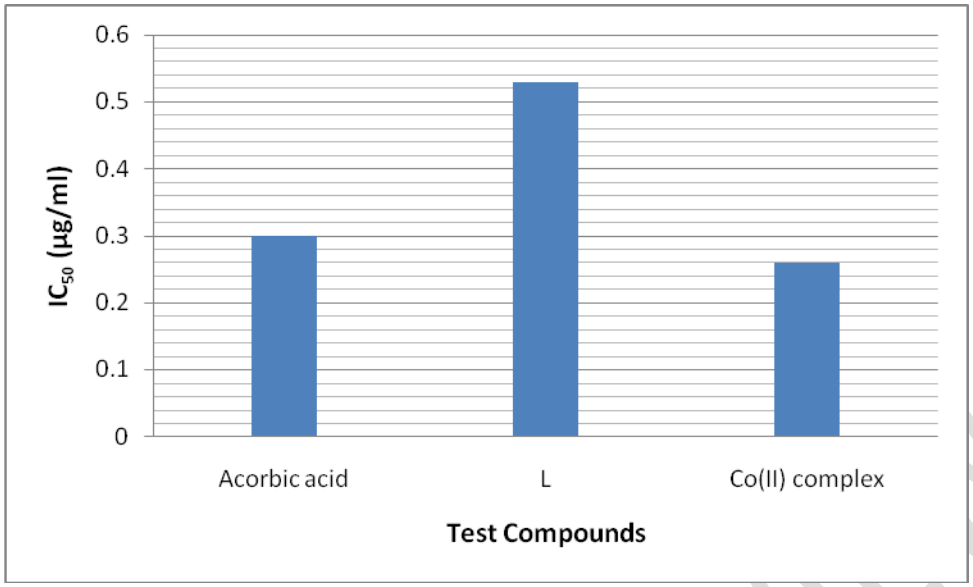


Figure 5: IC_{50} values of test compounds for NO radical scavenging activity

NO is readily oxidized to a peroxynitrite radical ($ONOO^{\cdot}$), which is a more stable radical in the presence of oxygen. However, protonation of peroxynitrite radical gives a highly reactive peroxynitrous acid (ONOOH) which has strong oxidizing properties toward various cellular constituents and can cause cell death, lipid peroxidation, carcinogenesis and aging [28]. An antioxidant compound which could scavenge rather than donating a hydrogen atom would be necessary to detoxify $NO^{\cdot}$. This prevents activating it to more cytotoxic radicals. The Co(II) complex at a low concentration (0.26µg/ml) has shown that it possesses the capacity of scavenging $NO^{\cdot}$. It is probably capable of coordinating $NO^{\cdot}$ as a ligand to form a stable chelate in a ring structure rather than donating hydrogen atom to $NO^{\cdot}$. This complex is therefore capable of reducing $NO^{\cdot}$ to a non toxic end product. The comparison of IC_{50} values of Co(II) complex with **L** and ascorbic acid shows that Co(II) complex has a better mechanism of scavenging NO radical. The is because the Co(II) ion present in this complex is capable of reducing $NO^{\cdot}$ to a non toxic metabolite.

The reducing power of an antioxidant is its ability to abstract or receive electrons from a free radical to form a more stable compound. Figure 6 therefore shows the comparison of the results of the reducing power activities of the tested compounds.

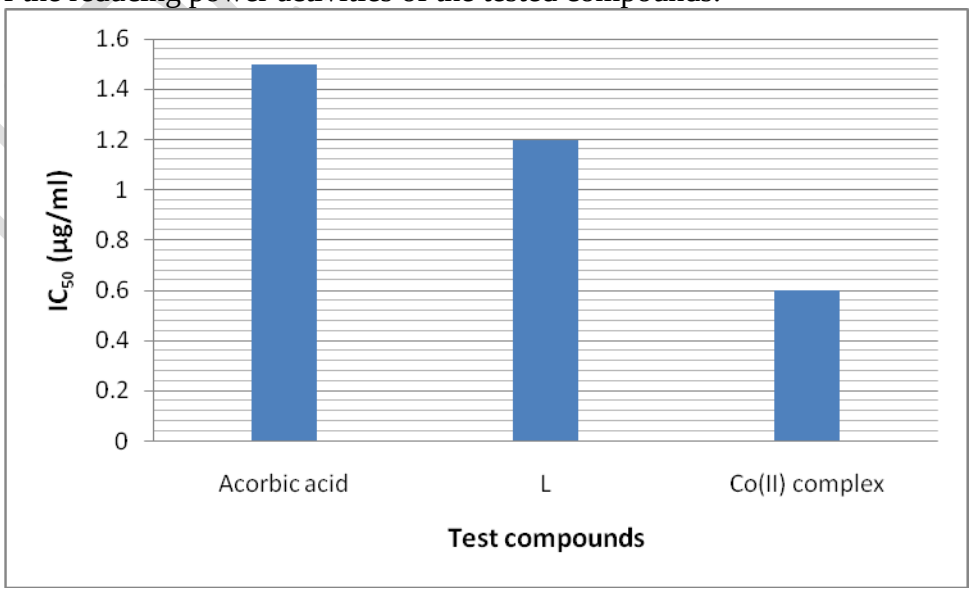


Figure 6: IC_{50} values of test compounds for reducing power activity of the test compounds

These results revealed that **L** and its Co(II) complex have very effective reducing power than ascorbic acid. Thus they could scavenge negatively charged free radicals at very low concentrations compared with ascorbic acid the reference antioxidant compound. Also, the Co(II) ion in the metal complex is capable of receiving the lone pairs of electrons on free radicals into its empty d-orbitals, thereby forming a new stable and harmless complex. This serves as an explanation for its effective reducing power.

4.0 CONCLUSION

Microwave irradiation technique has been successfully developed for the synthesis of 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one (**L**) and its Co(II) complex. The *in-vitro* antibacterial activities of **L** evaluated against three gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and Haemolytic *Staphylococcus aureus*) and three gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* sp.) revealed it was active against *Pseudomonas aeruginosa* with a very high zone of inhibition, about thrice that of tetracycline (clinical drug). The *in-vitro* antifungal activities of **L** evaluated against three fungi (*Aspergillus niger*, *Trichoderma viride* and *Penicillium citrinum*) revealed it was active against *Penicillium citrinum*. 1-Benzyl-3-[(4-methylphenyl)imino]-indolin-2-one has also proven to be a good antioxidant compound through its potent reducing ability, nitric oxide and DPPH radical scavenging activities. Test results showed it could scavenge relatively stable free radicals before they decompose into unstable and highly reactive radicals. However, its bactericidal effect might be through its hydrogen peroxide sparing effects. Hydrogen peroxide is a relatively unstable radical which easily decomposes to hydroxyl radical and finally oxygen and water. If hydrogen peroxide is spared in a living system, it is capable of initiating oxidative stress, DNA damage, abrogation of cellular functions and eventually cell death. As expected, the Co(II) complex has proven to be a better antioxidant compound than its ligand **L** and ascorbic acid through its potent reducing ability, nitric oxide and DPPH radical scavenging activities. It has a bi-dimensional approach to its activity: it confers protection to humans while being toxic to bacteria. However, it might be genotoxic and its genotoxicity is not within the scope of this research.

Conflict of Interests

The authors have no conflict of interests to declare.

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