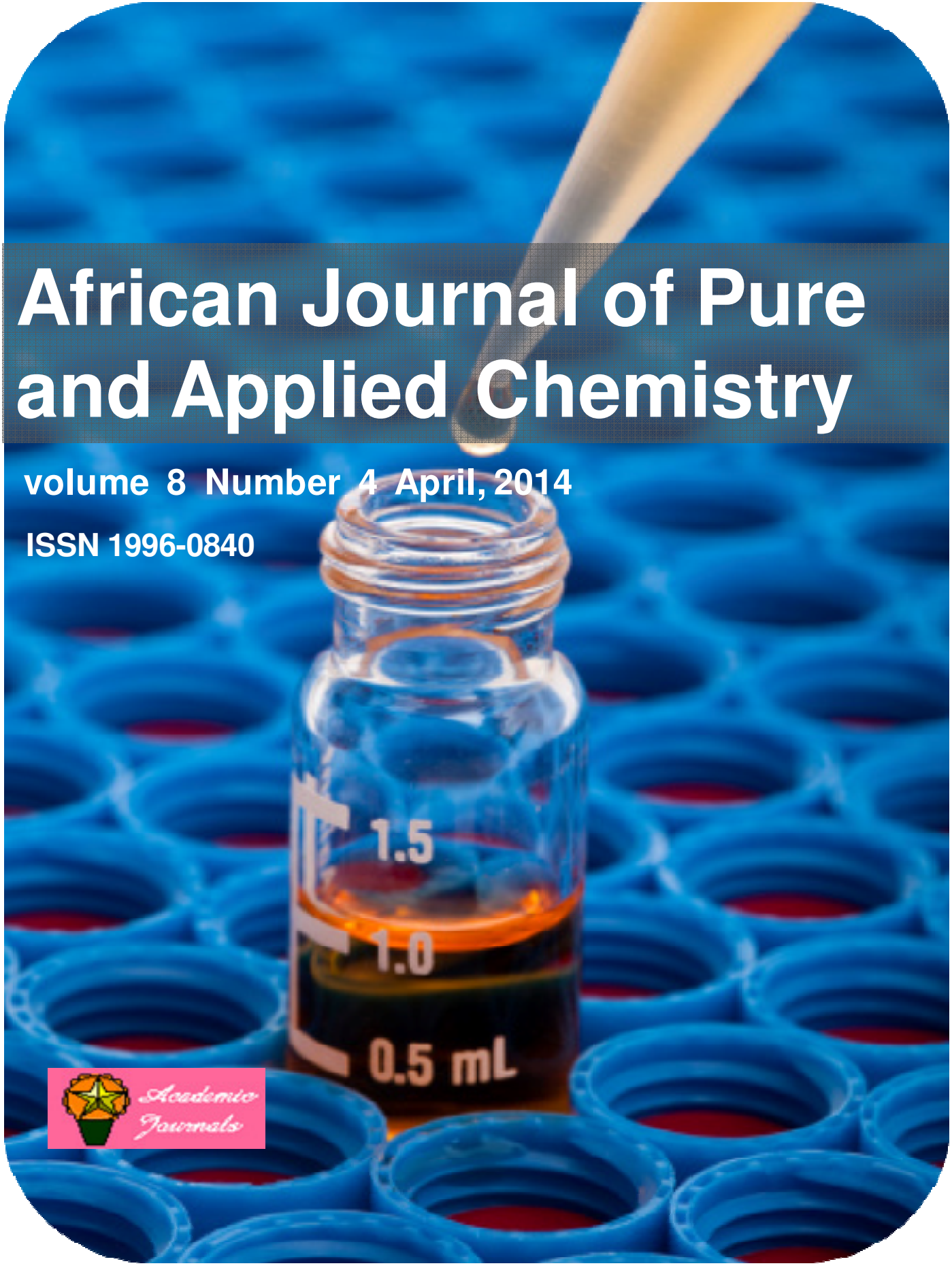

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Full Length Research Paper

1-Indanone chalcones and their 2,4-Dinitrophenylhydrazone derivatives: Synthesis, physicochemical properties and *in vitro* antibacterial activity

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Chalcones are natural biocides. Several publications appear every year covering the synthesis of chalcones because they exhibit an array of pharmacological activities. In this study, some condensation reactions of 1-Indanone with substituted benzaldehydes were carried out under different reaction conditions. The chalcone products were converted to their corresponding 2,4-Dinitrophenylhydrazone derivatives and evaluated against five gram-positive and eight gram-negative bacteria for their *in vitro* antibacterial property. Antimicrobial activity was observed against many of the tested strains, with zones of inhibition ranging from 10 to 28 mm. In many cases, the hydrazone derivatives were more active than their chalcone precursors. The best results were obtained against gram-negative bacteria for most of the compounds. Compound 1b was the most active with its minimum inhibitory concentrations (MICs) against six strains of bacteria ranging from 15.6 to 31.3 µg/ml, hence, could be developed as an antibacterial agent against infections caused by some gram-negative bacteria such as *Pseudomonas aeruginosa* and *Salmonella typhimurium*.

Key words: Antibacterial activity, chalcones, 2,4-Dinitrophenylhydrazones, 1-Indanone, microwave-assisted synthesis, minimum inhibitory concentration.

INTRODUCTION

A generic terminology for the 1,3-Diphenyl-2-propen-1-one moiety (**1)** is chalcone. Chalcones, which are found abundantly in edible plants, are considered to be intermediary compounds of biosynthetic route of flavonoids (**2)** and isoflavonoids (**3)** (Harborne, 1986; Akihisa et al., 2006; Zhang et al., 2006; Nowakowski, 2007).

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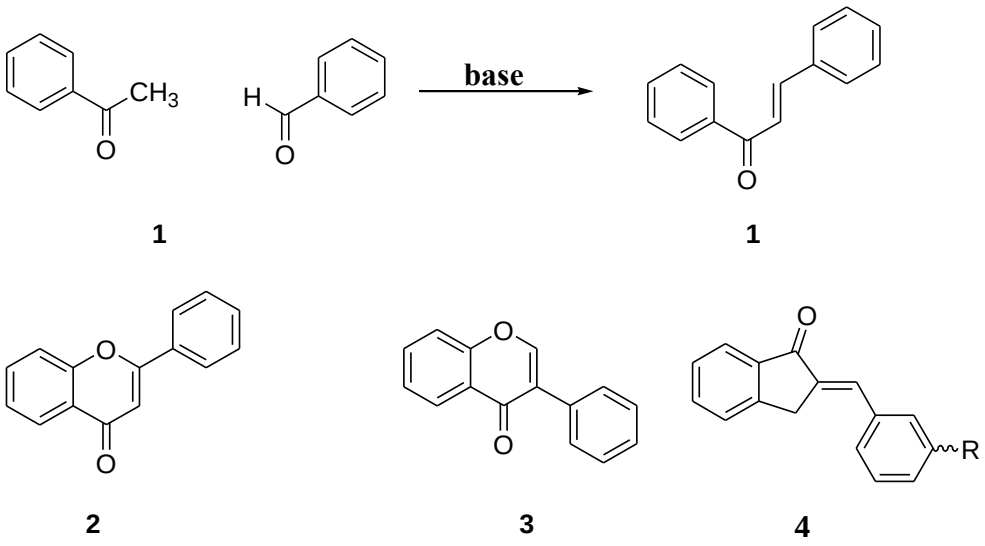


Scheme 1. Classical Claisen Schmidt condensation reaction
1, Chalcone; **2,** flavonoids; **3,** isoflavonoids; **4,** 2-(benzylidene)-1-indanones.

In many cases, chalcones serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects and herbivores (Vaya et al., 1997).

From the chemical point of view, they are α, β-Unsaturated ketones which are readily obtained via synthetic routes in the laboratory, by base-catalyzed condensation of aromatic (or heteroaromatic) aldehydes with an acetophenone (or its analogs) in aqueous alcohol (this is the classical Claisen Schmidt condensation reaction), (Furnis et al., 2004) (Scheme 1). Bases that have been used include NaOH, LiOH, $Ba(OH)_2$ and Na_2CO_3 in water (Zhang et al, 2003). They have been prepared also under ultrasound irradiation of alcohol solutions of mixtures of benzaldehydes and acetophenones, catalyzed by KOH and KF-Al_2O_3 (Li, 2002).

In addition, the chalcones can be prepared without the use of any solvent but using different catalysts, such as NaOH and KOH (by grinding) (Palleros, 2004; Zangade et al., 2011), NaOH on alumina (Sarda, et al., 2009), etc and under microwave irradiation on silica gel (Rajashree et al., 2013), iodine-alumina (Kakati and Sarma, 2011) and in the presence of $ZnCl_2$ (Sharma and Joshi, 2012). Furthermore, the synthesis of chalcones has been catalyzed by acids such as dry HCl (Széll and Sohár, 1969), $BF_3.OEt_2$ (Narender and Papi-Reddy, 2007), $RuCl_3$ (Iranpoor and Kazemi, 1998), silica-sulfuric acid and acetic acid-sulfuric acid (Thirunarayanan and Vanangamudi, 2007; Konieczny et al., 2007) and para-toluenesulfonic acid (solvent free and under microwave irradiation) (Gall et al., 1999).

A large number of articles reporting the bioactivity of chalcones appear every year. They have been reported to show a wide range of pharmacological activities, such as cytotoxic and chemoprotective (antioxidant, anti-invasive, inhibition of nitric oxide, inhibition and induction of metabolizing enzymes and inhibition of estrogen biosynthesis) (Go et al., 2005; Cheng et al., 2008), anti-trichomonal (Oyedapo et al., 2004), anti-tubercular (Hans et al., 2010), anti-inflammatory (Hsieh et al., 1998; Vogel et al., 2010), antimalarial [(Hans et al., 2010; Li et al., 1995; Mishra et al., 2008), antimicrobial (Pappano et al., 1994; Tomar et al., 2007; Sivakumar et al, 2009), etc.

Some work has been carried out on the biological property of 1-Indanone chalcones [2-(benzylidene)-1-indanones)] **4:** They have been shown to possess cytotoxic (Dimmock et al., 1999, 2002), *in vitro* antioxidant (Perjési and Rozmer, 2011; Huang et al., 2012), anti-cholinergic (Sheng et al., 2009), antifungal (Al-Nakib et al., 1997) and anticancer (Prakasham et al., 2012) activities.

Hydrazones (aryl-, heteroaryl, acyl and sulfonyl) have been reported to exhibit diverse biological properties such as anti-tubercular, anti-malarial, antimicrobial, anti-tumor, anti-inflammatory, anticonvulsant, analgesic, etc, activities and possess varied analytical applications (Rollas and Küçükgüzel, 2007; Ajani et al., 2010; de Oliveira et al., 2011; Suvarapu et al., 2012). It is pertinent to point out here that the development of drug resistance in human pathogens against commonly used antibacterial drugs, resulting in relapse of disease, has necessitated the search for new antimicrobial agents from both natural and synthetic sources. Screening of synthetic organic compounds for antimicrobial activities is important for finding potential new compounds for therapeutic use. The *in vitro* sensitivity testing of antibacterial agents against

pathogenic bacteria is very important because the results are useful in carrying out studies of animal models of infection (*in vivo* studies) (Greenwood, 1981). It has been pointed out that *in vitro* tests could not usually "be developed that would make it possible to (quantitatively) predict the efficacy of any antibiotic against any specific infection *in vivo*". The results of *in vivo* studies are utilized on the long run by practicing physicians to determine treatment recommendations for patients with infections (Greenwood, 1981; Zak et al., 1985).

In this paper, we wish to report the syntheses of 1-Indanone chalcone analogs using different reaction conditions, their corresponding 2,4-Dinitrophenylhydrazone derivatives and the assessment of their effect on some pathogenic bacteria.

MATERIALS AND METHODS

Chemistry

The purity of all described compounds was checked by melting point (m.p.) and thin layer chromatography (TLC) (E. Merck Kieselgel 60 F254).

Melting points (uncorrected) were determined using a gallenkamp melting point machine. R_f values were determined using silica gel F_{254} TLC plates (Merck), developed with n-Hexane:ethyl acetate (2:1) and observed under UV light (λ = 254 and 366 nm). The infrared (IR) spectra were recorded as KBr pellets using a Bruker IFS 13v Fourier transform infrared spectroscopy (FT-IR) spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 400 MHz AVANCE spectrometer. The data were obtained from $CDCl_3$ solutions. Chemical shifts are given in the δ scale (ppm) using tetramethylsilane as an internal standard. The elemental analysis (C, H, N) of the compound was performed using a Carlo Erba-1108 elemental analyzer.

Conventional base catalyzed synthesis of 1,3,3a,8a-Tetrahydro-1,3-diphenylspiro[cyclopent[a]indene-2(8H),2'-[2H]indene]-1',8(3'H)-dione (1a), (E)-2-[4-(dimethylamino)benzylidene]-2,3-dihydro-1H-inden-1-one (2a), (E)-2-(4-Methoxybenzylidene)-2,3-Dihydro-1H-Inden-1-one (3a) and (E)-2-(4-Hydroxy-3-Methoxybenzylidene)-2,3-Dihydro-1H-inden-1-one (6a)

40 mmol of the benzaldehyde derivative was added to a 2.5% methanolic solution of KOH (25 ml) in a conical flask while stirring. 1-Indanone (40 mmole) dissolved in methanol (20 ml) was then added dropwise to the basic reaction mixture. The reaction was left to stir for 18 h. at room temperature.

The product formed was filtered, washed with water, and dried in the oven followed by recrystallization from ethanol.

1a: IR (KBr; cm^{-1}): 1700 (C=O), 1603 (C=C), 1240, 1208, 1096, 1013, 911, 875. ^{1}H NMR ($CDCl_3$), ó ppm; 7.83 (d, 1H, J = 7.8 Hz, Ar); 7.59 (d, 1H, J = 7.4 Hz, Ar); 7.50 (m, 2H, Ar); 7.25 (m, 13H, Ar); 6.95 (d, 1H, J = 7.6 Hz, Ar); 4.63 (t, 1H); 4.16 (d, 1H); 3.99 (t, 1H); 3.89 (d, 1H); 3.09(dd, 2H, CH2). ^{13}C NMR (100 MHz,$CDCl_3$), ó ppm; 208.18 (C=O), 206.19 (C=O), 156.26 (C_q), 153.30 (C_q), 137.66 (C_q), 137.15 (C_q), 136.95 (C_q), 136.20 (C_q), 135.66, 135.16, 128.94, 128.80, 128.74, 128.64, 127.80, 127.51, 127.46, 126.23, 125.75, 124.94, 123.84, 70.67 (C_q), 59.70, 54.60, 53.45, 46.51, 30.04 (CH_2). Anal. (%) for $C_{32}H_{24}O_2$: Calcd. C, 87.25; H, 5.49; Found: C, 87.21; H, 5.52.

2a: IR (KBr; cm^{-1}): 1680 (C=O), 1596 (C=C), 1276, 1183, 1101, 957, 818. ^{1}H NMR ($CDCl_3$), ó ppm; 7.90 (1H, d, J = 7.0 Hz, Ar), 7.28-7.67 (m, 6H, Ar), 6.65 (2H, d, J = 7.6 Hz, Ar), 3.90 (s, 2H, CH_2), 3.00 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$), ó ppm; 194.70 (C=O), 151.50 (C_q), 149.86 (C_q), 139.15 (C_q), 135.38, 134.21, 133.20, 130.18 (C_q), 127.74, 126.44, 124.36, 123.49 (C_q), 112.27, 40.41 (CH_3), 33.10 (CH_2). Anal. (%) for $C_{18}H_{17}NO$: Calcd. C, 82.10; H, 6.51; N, 5.32; Found: C, 82.15; H, 6.52; N, 5.37.

3a: IR (KBr; cm^{-1}): 1695 (C=O), 1625, 1601 (C=C), 1258, 1185, 1100, 1025, 958, 924, 822. ^{1}H NMR ($CDCl_3$), ó ppm;). 7.87 (1H, d, J = 6.8 Hz, Ar), 7.50-7.59 (m, 5H, Ar), 7.38 (m, 1H, Ar), 6.94 (d, J = 8.4 Hz, 2H, Ar), 3.90 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$), ó ppm; 194.70 (C=O), 161.23 (C_q), 149.9 (C_q), 138.50 (C_q), 134.72, 134.12, 132.96, 132.77 (C_q), 128.47 (C_q), 127.93, 126.53, 124.59, 114.84, 55.76 (CH_3), 32.84 (CH_2). Anal. (%) for $C_{17}H_{14}O_2$: Calcd. C, 81.58; H, 5.64; Found: C, 81.61; H, 5.66.

6a: IR (KBr; cm^{-1}): 3490 (OH), 1677 (C=O), 1590 (C=C), 1249, 1201, 1167, 1101, 1037, 975. ^{1}H NMR ($CDCl_3$), ó ppm; 9.74 (s, 1H, OH), 7.75 (d, J = 7.7 Hz, 1H, Ar), 7.65-7.67 (m, 2H, Ar), 7.24-7.48 (m, 4H, Ar), 6.90 (d, J = 8.4Hz, 1H, Ar), 4.07 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$):, ó ppm; 194.07 (C=O), 150.63 (C_q), 149.87 (C_q, 148.69 (C_q), 138.46 (C_q), 135.28, 134.61, 132.55 (C_q), 128.38, 127.41, 127.35 (C_q), 125.92, 124.24, 116.85, 115.57, 56.53 (CH_3), 32.72 (CH_2). Anal. (%) for $C_{17}H_{14}O_3$: Calcd. C, 76.68; H, 5.30; Found: C, 76.61; H, 5.40.

Microwave assisted synthesis of (E)-2-(4-Hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (4a), (E)-2-(3-Hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (5a) and (E)-2-(4-Hydroxy-3-methoxybenzylidene)-2,3-dihydro-1H-inden-1-one (6a)

1-Indanone (7.60 mmol), the appropriate benzaldehyde (7.60 mmol) and silica gel (5.0 g) were mixed together and ground into a fine powder in a mortar. Acetic acid (5 drops) and concentrated H_2SO_4 (1 drop) were added and mixed thoroughly. The mixture was transferred into a beaker and irradiated in a microwave oven (medium power) for 6 min. After the irradiation, the mixture was extracted with hot ethanol (2 × 50 ml) and the filtered clear solution left to stand at room temperature until fine yellow crystals formed. The product was filtered and oven-dried.

4a: IR (KBr; cm^{-1}): 3420 (OH), 1685 (C=O), 1595 (C=C), 1245, 1195, 1100, 1035, 960. ^{1}H NMR ($CDCl_3$), ó ppm; 10.14 (s, 1H, OH), 7.75 (d, J = 7.2 Hz, 1H, Ar), 7.62 (m, 4H, Ar), 7.42-7.47 (m, 2H, Ar), 6.92 (d, J = 7.6 Hz, 2H, Ar), 4.00 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$), ó ppm; 194.07 (C=O), 160.30 C_q), 150.57 (C_q), 138.47 (C_q), 135.25, 134.23, 133.81, 132.39 (C_q), 128.38, 127.39, 126.90 (C_q), 124.24, 116.93, 32.82 (CH_2). Anal. (%) for $C_{16}H_{12}O_2$: Calcd. C, 81.34; H, 5.12; Found: C, 81.31; H, 5.16.

5a: IR (KBr; cm^{-1}): 3481 (OH), 1695 (C=O), 1601 (C=C), 1218, 1136, 1091, 922, 832. ^{1}H NMR ($CDCl_3$), ó ppm; 9.69 (s, 1H, OH), 7.18-7.78 (m, 8H, Ar), 6.89 (s, 1H), 4.05 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$), ó ppm; 194.18 (C=O), 158.60 (C_q), 150.82 (C_q), 138.11 (C_q), 136.94 (C_q), 135.69, 133.93, 130.83, 128.53, 127.53, 124.44, 122.86, 117.99, 117.86, 32.85 (CH_2). Anal. (%) for $C_{16}H_{12}O_2$: Calcd. C, 81.34; H, 5.12; Found: C, 81.35; H, 5.08.

Synthesis involving $SOCl_2$ catalyst for (E)-2-(4-Hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (4a), (E)-2-(3-Hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (5a) and (2E)-2-(2-Nitrobenzylidene)-2,3-dihydro-1H-inden-1-one (7a)

A mixture of 1-Indanone (7.6 mmol) and 1 mol equivalent of the

benzaldehyde derivative was added to 10 ml of absolute ethanol in a conical flask. 1.0 ml of thionyl chloride ($SOCl_2$) was added dropwise in a fume cupboard while stirring. The mixture was left to stir for 18 h. 50 ml of cold water was added to the reaction mixture with stirring to afford the precipitation of the product. This was filtered, washed with water and dried. Recrystallisation from ethanol gave the pure products.

7a: IR (KBr; cm^{-1}): 1675 (C=O), 1631 (C=C), 1318, 160, 1133, 892. ^1H NMR ($CDCl_3$), ó ppm; 8.05 (d, 1H, J = 8.1 Hz, Ar), 7.89 (s, 1H), 7.84 (d, 1H, J = 7.8 Hz, Ar), 7.54-7.67 (m, 4H, Ar), 7.45 (d, 1H, J = 6,8 Hz, Ar), 7.36 (m, 1H, Ar), 3.80 (s, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$), ó ppm; 193.57 (C=O), 150.00 (C_q), 149.23 (C_q), 138.54 (C_q), 138.22 (C_q), 135.48, 133.80, 131.56 (C_q), 131.02, 130.05, 129.82, 128.26, 126.70, 125.44, 124.99, 31.49 (CH_2). Anal. (%) for $C_{16}H_{11}NO_3$: Calcd. C, 72.45; H, 4.18; N, 5.28. Found: C,72.49; H, 4.16; N, 5.22.

Conventional method for synthesis of (E)-2-(4-Hydroxy-3-methoxybenzylidene)-2,3-dihydro-1H-inden-1-one 2,4-Dinitrophenylhydrazone (6b)

2,4-Dinitrophenylhydrazine (DNP) (4.0 mmol) was added to absolute methanol (50 ml), followed by concentrated H_2SO_4 (2 ml). One molar equivalent of Compound **6a** dissolved in absolute methanol (20 ml) was added to the DNP/methanol mixture in a round bottom flask and refluxed for 5 min with the colour changing from orange to deep red. The reaction mixture was allowed to cool and left to stand for 2 h. The product crystallized out gradually from solution and was separated by filteration and dried in the oven. IR (KBr; cm^{-1}): 3335 (NH), 1614 (C=N), 1512, 1334 (NO_2). ^1H NMR ($CDCl_3$) ó ppm; 11.77 (s, 1H, NH), 9.47 (s, 1H, OH), 8.85 (d, 1H, J = 2.6 Hz, Ar), 8.17-8.40 (m, 3H, Ar), 7.50-7.63 (m, 4H, Ar), 7.11-7.19 (m, 2H, Ar), 6.94 (d, 1H, J = 8.1 Hz), 4.06 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3). Anal. (%) for $C_{23}H_{18}N_4O_6$: Calcd. C, 61.88; H, 4.06; N, 12.55. Found: C,62.01; H, 4.00; N, 12.68.

Microwave assisted synthesis of 2,4-Dinitrophenylhydrazone derivatives of 1,3,3a,8a-Tetrahydro-1,3-diphenylspiro[cyclopent[a]indene-2(8H),2'-[2H]indene]-1',8(3'H)-dione (1b), (E)-2-[4-(Dimethylamino)benzylidene]-2,3-dihydro-1H-inden-1-one (2b), (E)-2-(4-Methoxybenzylidene)-2,3-dihydro-1H-inden-1-one (3b), (E)-2-(4-Hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (4b), (E)-2-(3-Hydroxybenzylidene)-2,3-dihydro-1H-inden-1-one (5b) and (2E)-2-(2-Nitrobenzylidene)-2,3-dihydro-1H-inden-1-one (7b)

2 mmol of DNP was dissolved in absolute methanol (40 ml) in a 250 ml beaker and 1 ml of concentrated H_2SO_4 was added. 1 mol equivalent each of compounds **1a, 2a, 3a, 4a, 5a, and 7a,** dissolved in 10 ml of absolute methanol was added to the DNP/methanol mixture and then pulse irradiated for 25 s. The products appeared within 15 s. The products were separated by filtration, washed with water, oven-dried and then recrystallized from ethanol.

1b: Yield 82%, m.p. 262 to 264°C. IR (KBr; cm^{-1}): 3311 (NH), 1695 (C=O), 1616 (C=N), 1500, 1334 (NO_2). ^1H NMR ($CDCl_3$) ó ppm; 10.01 (s, 1H, NH), 8.89 (s,1H, Ar), 8.25 (d, 1H, J = 9.8 Hz, Ar), 7.94 (m, 2H, Ar), 7.15-7.44 (m, 8H, Ar), 6.92-7.09 (m, 9H, Ar), 4.70 (t, 1H), 4.40 (t, 1H), 3.90 (dd, 2H, CH_2), 3.35 (d, 1H), 3.15 (d, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), ó ppm; 207.46 (C=O), 163.29 (C=N), 153,07, 150.01, 144.85, 138.27, 136.97, 136.86, 136.73, 135.26, 132.34, 129,87, 129.56, 129.32, 129.06, 128.87, 128.52, 128.44, 128.20, 127.85, 127.49, 127.44, 126.11, 124.83, 123.54, 117.28, 70.29, 60.43, 57.30, 50.70, 49.65, 30.13. Anal. (%) for $C_{38}H_{28}N_4O_5$: Calcd. C, 73.54; H, 4.55; N, 9.03. Found: C, 73.49; H, 4.46; N, 9.09.

2b: Yield 76%, m.p. 195 TO 197°C. IR (KBr; cm^{-1}): 3338 (NH), 1614 (C=N), 1589 (C=C), 1510, 1332 (NO_2). ^1H NMR ($CDCl_3$), ó ppm; ; 11.80 (s, 1H, NH), 8.90 (s, 1H, Ar), 8.29 (d, 1H, J = 9. 1 Hz, Ar), 6.80-8.21 (m, 10H, Ar), 4.01 (s, 2H, CH_2), 2.99 (s, 6H, $N(CH_3)_2$). Anal. (%) for $C_{24}H_{21}N_5O_4$: Calcd. C, 65.00; H, 4.77; N, 15.79. Found: C,65.19; H, 4.67; N, 15.68.

3b: Yield 94%, m.p. 213 to 214°C. IR (KBr; cm^{-1}): 3340 (NH), 1614 (C=N), 1589 (C=C), 1512, 1332 (NO_2). ^1H NMR ($CDCl_3$), ó ppm; 11.10 (s, 1H, NH), 9.01 (s, 1H, Ar), 8.24(d, 1H, J = 9.8 Hz, Ar), 7.30-7.88 (m, 8H, Ar), 6.96 (d, 2H, J = 8.2 Hz, Ar), 3.88 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3). Anal. (%) for $C_{23}H_{18}N_4O_5$: Calcd. C, 64.18; H, 4.22; N, 13.02. Found: C,64.37; H, 4.36; N, 13.23

4b: Yield 67%, m.p. 225 to 226°C. IR (KBr; cm^{-1}): 3346 (NH), 1612 (C=N), 1589 (C=C), 1510, 1330 (NO_2). ^1H NMR ($CDCl_3$), ó ppm; 11.56 (s, 1H, NH), 10.05 (s, 1H, OH), 8.99 (s, 1H, Ar), 8.28 (d, 1H, J = 9.4 Hz, Ar), 8.00 (d, 1H, J = 9.8 Hz, Ar), 7.40-7.80 (m, 7H, Ar), 6.94 (d, 2H, J = 8.3 Hz, Ar), 3.98 (s, 2H, CH_2). Anal. (%) for $C_{22}H_{16}N_4O_5$: Calcd. C, 63.46; H, 3.87; N, 13.46. Found: C,63.32; H, 3.91; N, 13.53

5b: Yield 53%, m.p. 244 to 245°C. IR (KBr; cm^{-1}): 3338 (NH), 1608 (C=N), 1590 (C=C), 1510, 1330 (NO_2). ^1H NMR ($CDCl_3$), ó ppm; 11.66 (s, 1H, NH), 9.98 (s, 1H, OH), 9.05 (s, 1H, Ar), 8.29 (d, 1H, J = 9.6 Hz, Ar), 7.20-8.01 (m, 9H, Ar), 6.89 (s, 1H, Ar), 4.05 (s, 2H,CH_2). Anal. (%) for $C_{22}H_{16}N_4O_5$: Calcd. C, 63.46; H, 3.87; N, 13.46. Found: C,63.50; H, 3.95; N, 13.50

7b: Yield 87%, m.p. 294 to 297°C. IR (KBr; cm^{-1}): 3329 (NH), 1613 (C=N), 1597 (C=C), 1501, 1334 (NO_2). ^1H NMR ($CDCl_3$), ó ppm; 10.89 (s, 1H, NH), 9.01 (s, 1H, Ar), 8.28 (d, 1H, J = 9.8 Hz, Ar), 8.01-8.06 (m, 2H, Ar), 7.87 (s, 1H, Ar), 7.35-7.86 (m, 7H, Ar), 4.01 (s, 2H, CH_2). Anal. (%) for $C_{22}H_{15}N_5O_6$: Calcd. C, 59.33; H, 3.39; N, 15.72. Found: C,59.41; H, 3.38; N, 15.84

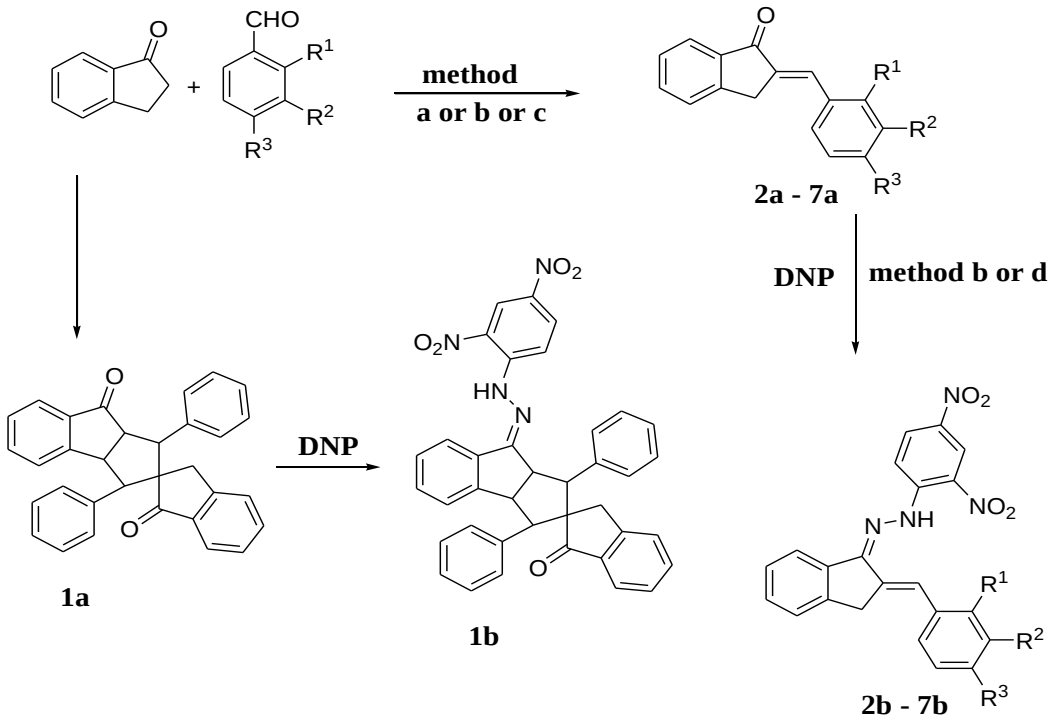
Microbiology: Antibacterial sensitivity testing

The antibacterial activities of the compounds were determined using the agar-well diffusion method as described by Russell and Furr (1977) and Akinpelu and Kolawole (2004). The test micro-organisms used in this study were obtained from the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory, University of Fort Hare, Alice, South Africa, and include the following:

Escherichia coli (ATCC 8739), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Staphylococcus aureus* (ATCC 6538), *Streptococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC10702), *Bacillus pumilus* (ATCC 14884), *P. aeruginosa* (ATCC 7700), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 10031), *K. pneumoniae* (ATCC 4352), *Proteus vulgaris* (ATCC 6830), *P. vulgaris* (CSIR 0030), *Serratia marcescens* (ATCC 9986), *Acinetobacter calcoaceticus* (UP), *A. calcoaceticus anitratus* (CSIR), *K. pneumoniae* (LIO), *Bacillus subtilis* (LIO), *Shigella dysenteriae* (LIO), *Staphylococcus epidermidis* (LIO), *P. aeruginosa* (LIO), *P. vulgaris* (LIO), *Enterococcus faecalis* (LIO), *S. aureus* (LIO) *Micrococcus kristinae* (LIO) and *Micrococcus luteus* (LIO).

Minimum inhibitory concentration (MIC)

The MICs of the compounds and the reference antibiotic were



Scheme 2. Synthesis of 1-Indanone chalcones and hydrazone derivatives.
1a: $R^1 = R^2 = R^3 = H$ **1b:** $R^1 = R^2 = R^3 = H$
2a: $R^1 = R^2 = H$; $R^3 = N(CH_3)_2$ **2b:** $R^1 = R^2 = H$; $R^3 = N(CH_3)_2$
3a: $R^1 = R^2 = H$; $R^3 = OCH_3$ **3b:** $R^1 = R^2 = H$; $R^3 = OCH_3$
4a: $R^1 = R^2 = H$; $R^3 = OH$ **4b:** $R^1 = R^2 = H$; $R^3 = OH$
5a: $R^1 = H$; $R^2 = OH$; $R^3 = H$ **5b:** $R^1 = H$; $R^2 = OH$; $R^3 = H$
6a: $R^1 = H$; $R^2 = OCH_3$; $R^3 = OH$ **6b:** $R^1 = H$; $R^2 = OCH_3$; $R^3 = OH$
7a: $R^1 = NO_2$; $R^2 = R^3 = H$ **7b:** $R^1 = NO_2$; $R^2 = R^3 = H$
Method a: KOH/MeOH, rt (used to synthesize 1a - 3a, 6a); **Method c:** $SOCl_2$/ethanol (used to synthesize 4a, 5a, 7a).
Method b: Microwave-assisted synthesis (used to synthesize 4a - 6a, 1b - 5b, 7b); **Method d:** conventional heating (used to synthesize 6b)

determined using the method of Akinpelu and Kolawole (2004). Dimethyl sulfoxide was used as negative control. Two milliliter of different concentrations of the test solution was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentration regimes of 0.0313 and 2.0 mg/ml for the test compound and 0.0157 and 1.0 mg/ml for the standard antibiotics. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry under a laminar flow before streaking with 18 h old bacterial cultures. The plates were later incubated at 37°C for up to 72 h after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration of the test compound and standard antibiotics that will prevent the growth of the susceptible test bacteria.

RESULTS AND DISCUSSION

Chemistry

The 1-Indanone chalcones (**2a** - **7a**) were synthesized by a Claisen-Schmidt type of reaction in which various substituted benzaldehydes were reacted with 1-Indanone, using acid or base catalysts (Scheme 2). Benzaldehydes with hydroxyl substituents gave very low yields of the corresponding indanone chalcones when the conventional base catalyzed (methanolic KOH) condensation method was used, hence acid catalyzed procedure was employed for the synthesis of the chalcones. Compound **6a** was synthesized in high yield using microwave assisted method in the presence of a mixture of acetic acid and conc. H_2SO_4. This method, however, did not work well for the synthesis of Compounds **4a** and **5a** (where charring was observed). A different method was then employed for the synthesis of the two compounds, whereby HCl (generated *in situ* by adding a small quantity of $SOCl_2$ (thionyl chloride) to the alcoholic reaction medium) was used as a catalyst.

The base-catalyzed reaction of benzaldehyde with 1-Indanone yielded the dimer of the expected chalcone(**1a)**, as reported from the reaction under basic condition (Berthelette et al., 1997).

Table 1. Physicochemical properties and catalytic method of synthesis of the 1-Indanone chalcones.

Compound	Molecular formula	Molecular weight (g/mol)	Catalysis/Method	Colour	Melting point (°C)
1a	$C_{32}H_{24}O_2$	440.53	Base/Stirring (RT)	Cream	234-236
2a	$C_{18}H_{17}NO$	263.33	Base/Stirring (RT)	Yellow	166-167
3a	$C_{17}H_{14}O_2$	250.29	Base/Stirring (RT)	Cream	139-140
4a	$C_{16}H_{12}O_2$	236.26	Acid/$SOCl_2$/EtOH	Orange	225-226
5a	$C_{16}H_{12}O_2$	236.26	Acid/$SOCl_2$/EtOH	Off-white	196-198
6a	$C_{17}H_{14}O_3$	266.29	Base/Stirring (RT)	Yellow	187-188
6a	$C_{17}H_{14}O_3$	266.29	Silica/Acid/MW	Yellow	187-188
7a	$C_{16}H_{11}NO_3$	265.26	Acid/$SOCl_2$/EtOH	Off-white	165-166
1b	$C_{38}H_{28}N_4O_5$	620.65	Methanol/Acid/MW	Orange	262-264
2b	$C_{24}H_{21}N_5O_4$	443.46	Methanol/Acid/MW	Brown	195-197
3b	$C_{23}H_{18}N_4O_5$	430.41	Methanol/Acid/MW	Brown	213-214
4b	$C_{22}H_{16}N_4O_5$	416.39	Methanol/Acid/MW	Brown	225-226
5b	$C_{22}H_{16}N_4O_5$	416.39	Methanol/Acid/MW	Red	244-245
6b	$C_{23}H_{18}N_4O_6$	446.41	Methanol/Acid/Reflux	Black	225-227
7b	$C_{22}H_{15}N_5O_6$	445.39	Methanol/Acid/MW	Red	294-297

The corresponding chalcone hydrazones were synthesized from the condensation reaction of the chalcones with 2,4-DNP (2,4-) in methanol using conventional heating or microwave irradiation. The reaction of 2,4-DNP with the chalcones was rapid (30 s to 5 min). Only one of the carbonyl functional groups of Compound **1a** reacted with the 2,4-DNP. The second carbonyl functional group was not assesible for the nucleophile (2,4-DNP) due to steric hindrance. The physicochemical properties of the compounds are listed in Table 1.

All compounds have been characterized on the basis of spectral analysis (^{1}H-NMR, ^{13}C-NMR and IR) and elemental analysis. Assignments of ^{13}C-NMR resonances of the compounds were deduced from the analysis of the Attached Proton Test (APT) and Distortionless Enhancement by Polarization Transfer (DEPT) experiments. The spectroscopic data for all the synthesized compounds are in agreement with their structures. In the ^{1}H-NMR spectra, the signals for the aromatic protons appeared in the region δ 6.65-8.05 ppm, while those of the methylene protons (CH_2) of the indanone ring showed at δ 3.80 to 4.07 ppm. The ^{1}H-NMR spectrum of Compound **1a** was similar to the data reported by Berthelette et al. (1997).

The ^{13}C-NMR spectra of the chalcones similarly gave expected signals and the expected number of carbon atoms (CH, CH_2, CH_3 and quatenary carbons). The signal for the –CH_2- carbon of the indanone moiety appeared at around δ 32.00 ppm. The carbonyl carbon appeared around δ 194.00 ppm for all the compounds except Compound **1a** that showed two signals for the C=O at δ 208.18 and δ 206.19 ppm, while the spiro-carbon signal showed at δ 70.67 ppm.

In the IR spectra of the chalcones, the OH group showed around 3455 to 3491 cm^{-1} as a broad band, while absorption bands showed between 1665 and 1700 cm^{-1} for the C=O functional group and between 1573 and 1625 cm^{-1} for the C=C functional group.

Antimicrobial evaluation

The antimicrobial susceptibility tests of the 1-Indanone chalcones and their corresponding 2,4-dinitrophenylhydrazone derivatives were performed using the agar-well diffusion method against thirteen strains of gram-positive and gram-negative bacteria. The activity of the compounds against the microorganisms was assessed through the zone of inhibition and the MICs, whose values are shown in Table 2. A known antibiotic (tetracycline) was used for comparison.

The results showed that the compounds at a concentration of 200 µg/ml showed zones of inhibition ranging from 10 to 28 mm. The results further indicated that the fourteen synthesized compounds showed antimicrobial activity against *P. aeruginosa, Salmonella typhimurium* and *Shigella flexineri* (all gram-negative) strains. The MICs of the compounds against the *P. aeruginosa* strain ranged from 16.5 to 250 µg/ml and from 16.5 to 1000 µg/ml for *S. typhimurium* and *S. flexineri* strains.

In many cases, the hydrazone derivatives appear to be more active than their chalcone precussors, except for Compound **3** and in some other cases (Table 2). Compound **1b** exhibited the best activity with the lowest MIC values for four gram-negative bacterial strains (15.6 µg/ml) and two gram-positive strains (31.3 µg/ml).

Table 2. Minimum inhibitory concentrations (µg/ml) of the chalcones, derivatives and standard antibiotic.

Microoganisms	Gram-positive														
	1a	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**	**Tet**
Staphylococcus aureus (ATCC 6538)	125	31.3	250	ND	125	250	250	62.5	125	62.5	125	1000	1000	500	125
Bacillus cereus (ATCC 10702)	1000	ND	ND	1000	ND	1000	ND	1000	250	1000	ND	1000	1000	500	250
Enterococcus faecalis (LIO)	ND	100	ND	1000	1000	ND	1000	ND	ND	ND	1000	62.5	125	1000	500
Micrococcus luteus (LIO)	500	31.3	ND	1000	1000	ND	31.3	1000	ND	1000	1000	1000	1000	31.3	20
Bacillus pumilus (ATCC 14884)	ND	ND	ND	ND	ND	ND	250	ND	500	62.5	ND	ND	125	500	1000
	Gram-negative														
	1a	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**	**Tet**
Escherichia coli (ATCC 25922)	ND	500	1000	ND	ND	1000	1000	ND	ND	625	ND	1000	ND	31.3	130
Pseudomonas aeruginosa (ATCC 15442)	62.5	15.6	250	125	125	250	15.6	250	250	15.6	62.5	31.3	125	3 1.3	ND
Salmonella typhimurium (ATCC 13311)	250	15.6	250	62.5	31.3	250	125	62.5	250	1000	250	31.3	125	31.3	ND
Klebsiella pneumonia (ATCC 4352)	ND	ND	ND	ND	ND	ND	ND	ND	125	ND	ND	ND	125	ND	ND
Proteus vulgaris (CSIR 0030)	125	500	ND	1000	31.3	ND	62.5	125	ND	1000	62.5	ND	500	500	16000
Serratia marcescens (ATCC 9986)	250	500	ND	62.5	62.5	ND	62.5	125	ND	ND	15.6	31.3	62.5	500	250
Shigella flexineri (LIO)	500	15.6	250	62.5	62.5	250	125	1000	250	1000	500	1000	125	15.6	250
Acinetobacter calcaoceuticus anitratus (CSIR)	125	15.6	250	125	62.5	1000	ND	125	500	ND	62.5	62.5	62.5	ND	250

Tet = tetracycline; ATCC = American Type Culture Collection; LIO = Locally Isolated Organism.

Comparing the activities of the compounds against the strains of bacteria, we can notice that they were globally more active against the gram-negative bacteria. *P.* aeruginosa is the most sensitive organism to the synthesized compounds and the activity order is **4a > 6a = (1a) >3a = 7a > 2a = 5a** for the chalcones and (**1b) = 5b > 6b =7b > 2b > 3b = 4b** for their corresponding hydrazones.

In the past several years, there has been an increasing use of quantitative structure-activity relationship (QSAR) to predict the biological activities of various organic molecules. One method that has been extensively employed involves the QSAR approach together with multivariate data analysis, combined with statistical design (Vasanthanathan et al., 2006). This approach is an attempt to show that there is a relationship between biological activities of compounds and structural or molecular descriptors such as physicochemical, thermodynamic, electronic, topological or geometrical parameters (Podunavac-Kuzmanović et al., 2008). The importance of the lipophilic or hydrophobic nature of bioactive compounds has been brought into fore by the tremendous progress in the use of QSAR methods. The penetration of bioactive compounds through the apolar cell membranes is modified by lipophilicity, characterized by the partition coefficient (log P). A measure of hydrophobicity/lipophilicity is the octanol/water partition coefficient Clog P.

In this work, Clog P was calculated using ChemDraw Ultra 8.0 software (CambridgeSoft Corporation). The results obtained are given in Table 3. The calculated values of log P for the hydrazones were higher than for the corresponding chalcones. However, the dimeric Compounds **1a and 1b** have the highest values. Taking the calculated lipophilicity (Clog P) values for the hydrazones and comparing with their corresponding activity (MIC) values for the most sensitive organism, *P. aeruginosa*, it could be seen that they are not of the same increasing order:

Clog P: **1b > 2b > 3b > 7b > 4b = 5b >6b**
MIC (*P. aeruginosa*)-activity: **1b = 5b > 6b = 7b >**

Table 3. Calculated Clog P and MR values.

Compound	Log P	CLog P	MR (cm^3/mol)	CMR
1a	6.52	5.758	134.93	13.389
2a	3.79	3.764	85.65	8.488
3a	3.38	3.518	77.72	7.809
4a	3.12	2.932	72.28	7.345
5a	3.12	2.932	72.28	7.345
6a	2.99	2.781	79.53	7.962
7a	2.90	3.342	-	7.803
1b	7.14	8.475	-	17.951
2b	5.89	6.923	-	13.051
3b	5.19	6.677	-	12.371
4b	4.67	6.091	-	11.908
5b	4.67	6.091	-	11.908
6b	4.80	5.940	-	12.524
7b	5.02	6.501	-	12.366

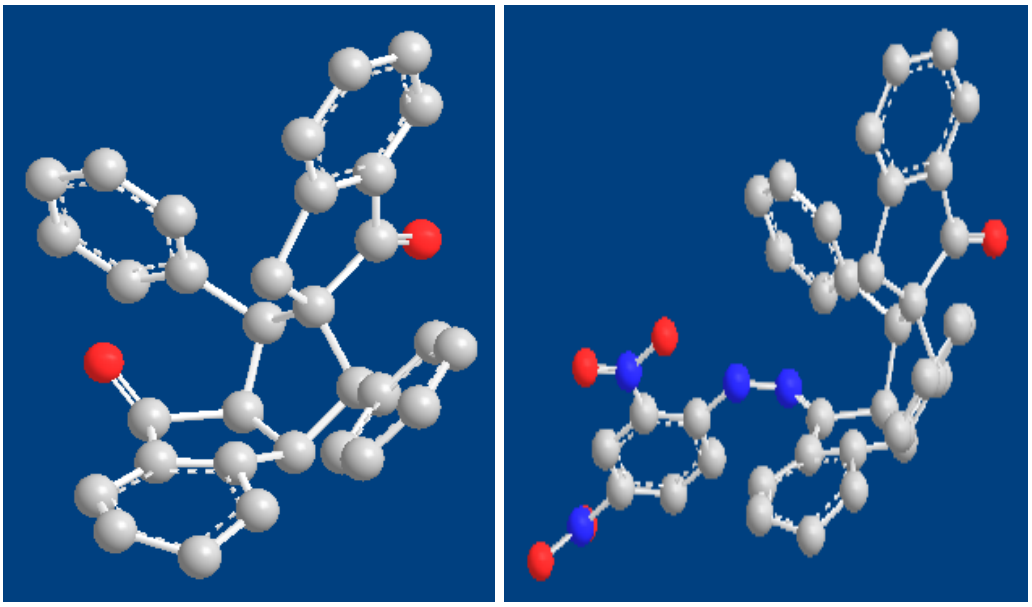


Figure 1. Three dimensional structure of Compounds **1a** and **b,** respectively.

2b > 3b = 4b

For all the structures synthesized, no significant correlation could be established between [pMIC = -log(MIC)] and log(P).

In a similar manner, the molar refractivity (MR - which represents size and polarizability) describing steric effects was calculated using ChemDraw Ultra 8.0 software and the results are included in Table 3. The largest molecule having the largest CMR and log P values (**1b)** has the best antimicrobial activity. This compound may be viewed as having a cup-like structure in which the bulky phenyl groups form the rim of the cup (Figure 1).

Conclusion

Different methods have been successfully employed in the synthesis of some 2-Arylidene-1-indanone derivatives in good yields. The compounds have been characterized using IR, ^{1}H and ^{13}C NMR and elemental analysis and their effects on some pathogenic bacteria evaluated. They exhibited broad spectrum antibacterial activity.

However, the compounds are more active against the gram-negative bacteria. The 2,4-Dinitrophenylhydrazone derivative of the dimer of 2-Benzylidene-1-indanone, **1b**, exhibited the highest antibacterial activity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Determination of some heavy metals in selected edible vegetables grown along River Yedzaram in Uba area Adamawa State, Nigeria

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The levels of some heavy metals were investigated in selected edible potions of the vegetables; *Amarathus caudatus* (Spinach) and *Hibiscus sabdariffa* (Rosella) are grown in Uba area along the Yedzaram River in North Eastern Adamawa State, Nigeria. All samples were randomly collected from two different gardens. The levels of the heavy metals, (Cu, Fe, Cd, Cr and Zn) were analyzed using Atomic Absorption Spectrophotometer (AAS) (BUK 210 model). In all the samples analyzed, Cd and Cr were not detected. The levels of heavy metals in Farm A for *H. sabdariffa* leaves ranges from Cu (30.00 ± 0.15 mg/kg to 31.00 ± 0.18 mg/kg), Fe (37.39 ± 0.02 mg/kg to 48.47 ± 0.10 mg/kg), Zn (13.00 ± 0.01 mg/kg to 25.50 ± 0.48 mg/kg), respectively. In Spinach, the results ranged from Cu (34.33 ± 0.42 mg/kg to 34.50 ± 0.05 mg/kg), Fe (31.72 ± 0.71 mg/kg to 43.33 ± 0.02 mg/kg), Zn (21.17 ± 0.14 mg/kg to 10.83 ± 0.17 mg/kg), respectively. The data were analyzed with t-test and analysis of variance (ANOVA). There were significant differences ($p < 0.05$) between the levels of the heavy metals in the vegetables obtained from Farms A and B. The order of the metal contamination in the vegetables was Fe > Cu > Zn in Farm A and Cu> Fe> Zn in Farm B. The elevated levels of metals in vegetables in the two gardens could be attributed to excessive usage of fertilizers and other agro - chemicals and of course the environmental factors of the areas. The results were however lower than the published threshold values considered toxic for mature plant tissue, except Fe which has higher values. The consumption of these vegetables as food may not pose possible health hazards to human at the time of the study.

Key words: Heavy metals, *Amarathus caudatus*, *Hibiscus sabdariffa*, Uba area, fertilizers.

INTRODUCTION

Vegetable is a plant or part of a plant used as food, typically as accompany to meat or fish, such as cabbage, potato, carrot or beans (Ihekoroye and Ngoddy, 1985). Eating vegetables regularly in diet can have many health

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benefits by reducing many health related diseases and used to convert the fats and carbohydrates into energy (Milk, 2012). Eating vegetables is one of the most important pathways for the human body to absorbed dietary mineral, necessary for its healthy developmentbut unfortunately harmful element such as heavy metals which may lead to intoxication and with prolong accumulation are being found in these vegetables (Elsevier, 2008).

However, heavy metals concentrations in soil are associated with biological and geochemical cycles and are influenced by anthropogenic activities such as agricultural practices, industrial activities and waste disposal methods (Ndiokwere and Ezeh, 1990; Usman and Ayodele, 2002; Uwah et al., 2009). Contamination and subsequent pollution of the environment by heavy metals have become a global concern due to their distribution and multiple effects on the ecosystem (Nriagu, 1990). Heavy metals are present in agricultural soils at low levels. Due to their cumulative behaviours and toxicity, they have potential, hazardous effect not only on plants but on human health (Das *et al.,* 1997). Distributions of heavy metals in plants depend upon availability and concentration of heavy metals as well as particular plant species (Punz and Seighardt, 1993). Many researchers have shown that some common vegetables are capable of accumulating high levels of metals from the soil (Xiong, 1998; Uwah et al., 2009). Certain species of Brassica (cabbage) are hyper-accumulators of heavy metals in their edible tissues (Xiong, 1998). Many people could be at risk of adverse health effects from consuming common vegetable cultivated in contaminated soil (Nirmal et al., 2007).

The populations mostly affected by heavy metals toxicity are pregnant women or very young children (Boon and Soltanpour, 1992). Neurological disorders, central nervous system (CNS) destruction and cancers of various body organs are some of the report of heavy metals poisoning (Agency for Toxic Substance and the Disease Registry (ATSDR) 1999a, b: 2000). Low birth weight and severe mental retardation of newly born children have been reported in some cases where pregnant women ingest toxic amount of heavy metal through direct or indirect means (Mahaffey et al., 1981).

Heavy metals like Fe, Cu, Zn, and Ni, are important for proper functioning of biological systems and their deficiency or excess could lead to a number of disorders (Ward, 2005; Uwah et al., 2009). Industrial, urban wastes and agricultural application and also mining activities results in an increased concentration of heavy metals in both soil and plant. Heavy metals pollute both soil and plant and so it is necessary to examine the state of the polluted soil and plant and establish what influence heavy metals have on both. Heavy metals have great significance due to their toxicity and accumulative behaviour or and are not biodegradable (Shinggu et al., 2007). Surface soil may act as carriers and possible source of pollution, since the mobility of these metals is such that remain in upper layers without regard to soil type.

Moreover, these metals are not permanently fixed and can be released by changes in climatic or environmental condition such as rainfall (Nriagu and Pacyna, 1990). The main sources of roadside contaminants are the deposition of aerosol particles which are adhesive in nature. But in the urban environment, these particles originate mainly from road traffic, welding, emission from industries, construction activities and flaking of paint (Radojavice and Bashkin, 1999).

The use of polluted water in the immediate surroundings of big cities for growing of vegetables is a common practice in Nigeria. Although this water is considered a rich source of organic matter and plant nutrients, it also contains sufficient amount of soluble salts and metal like Fe, Mn, Cu, Zn, Pb, Ni, Sn, Hg, Cr, As and Al. When such water is used for irrigation of crops for a long period, these heavy metals may accumulate in soil and may be toxic to the plants and also cause deterioration of soil (Kirkham, 1983; Uwah, 2009).

Heavy metals contamination and pollution of environment has become a global concern, due to their distribution and multiple effects on the ecosystem, waste waters are highly use in agricultural irrigation and long-term usage of these waste waters on agricultural lands often results in the build-up of elevated levels of heavy metals in soils (Rattan et al., 2001). Crops usually cultivated on the metals contaminated soils accumulate these metals in excessive quantities are enough to cause clinical problems both to animal and human beings consuming these metals rich plants.

The study is aimed at investigating the levels of some heavy metals such as (Cu, Cd, Fe, Cr and Zn) in edible portions of spinach *(Amarathus caudatus)* and Rosella leaves *(Hibiscus sabdariffa),* cultivated along River Yedzaram in Uba area. Extrapolate the results and ascertained the suitability or otherwise of the vegetables for human consumptions.

This was carried out by analyzing spectrophotometrically the levels of the metals in the vegetable samples.

MATERIALS AND METHODS

Analytical reagent (AnalaR) grade chemicals and distilled water were used throughout the study. All glassware and plastic containers used in this work were washed with detergent solution followed by 20% (v/v) nitric acid and then rinsed with tap water and finally with distilled water.

Study area

Uba region geographically is located in the North-Eastern part of Borno State and Adamawa State, in North-eastern Nigeria. Its geographical coordinates are 10° 27' North and 13° 17' East of the Greenwich meridian. Uba region occupies land area of 2,362 km^2 and a population of 138,091 (Wandeo, 2005).

Table 1. Concentrations of some heavy metals in Spinach and Rosella from Farm A (mg/kg).

Vegetables/Sampling sites	Cd	Cr	Cu	Fe	Zn
Rosella (*Hibiscus sabdariffa*)					
Location 1	ND	ND	31.00 ± 0.18	48.67 ± 0.1	13.00 ± 0.01
Location 2	ND	ND	30.00 ± 15.00	37.39 ± 0.02	25.50 ± 0.48
Spinach (*Amaranthus caudatus*)					
Location 1	ND	ND	34.33 ± 0.42	43.33 ± 0.02	10.83 ± 0.17
Location 2	ND	ND	34.50 ± 0.05	31.72 ± 0.71	21.17 ± 0.14
WHO/FAO			20 - 100	10 -18	3 - 20
NAFDAC			0 - 40	10 - 20	0 - 50

All values represent mean ± standard deviation of triplicate determination. **ND =** Not detected**, WHO** (1996) **=** World Health organization, **NAFDAC** = National Agency for Food and Drug Administration and control.

Sampling and sample treatment

The samples analyzed include *H. Sabdariffa* (Spinach) and *A. caudatus* (Rosella) leaves. Samples were collected from May to September, 2012 from two different Farms (A and B) along the River Yedzaram Uba area. Edible portions of the fresh samples of *A. caudatus* (Rosella) and *H. sabdariffa* (Spinach) were randomly collected (handpicked) from two different vegetable Farms (A and B), which supply most of the vegetables consumed in Uba. The samples were wrapped in big brown envelopes and labeled. Only fresh vegetables in good conditions were collected in order to produce good quality dried product (Audu and Lawal, 2005). A total of 10 samples each of *A. caudatus* and *H. sabdariffa* from each of the vegetable farms along River Yadzaram in Uba were collected. Samples from each of the two farms were pooled together to obtained two homogenous samples.

In the laboratory, vegetable samples were washed with tape water and thereafter with distilled water and the water was allowed to drip out and were then sliced into smaller portion and then dried in an oven at 80°C for hours (AOAC, 2000). At the end of the drying, the oven turned off and left overnight to enable the sample cool to room temperature. Each sample was grounded into a fine powder, sieved and finally stored in a 250 cm^3 screw capped plastic jar appropriately labeled (AOAC, 2000).

Digestion procedure

1.0 g of each powdered leaves samples were weighed out into Kjeldahl digestion flask mixed with 10 cm^3 of concentrated sulphuric acid, concentrated perchloric acid and concentrated nitric acid in the ratio 1: 2: 20 by volume respectively and left to stand overnight. Thereafter, the flask was heated at 70°C for 40 min and then, the heat was increased to 120°C. The mixture turned black after a while (Jeffery et al., 1989). The digestion was completed when the solution became clear and white fumes appeared. The digest was diluted with 20 cm^3 of distilled water and boiled for 15 min. This was then allowed to cool, transferred into 100 cm^3 volumetric flasks and diluted to the mark with distilled water. The sample solution was then filtered through a filter paper into a screw capped polyethylene bottle.

Determination of heavy metals

Levels of Cd, Cu, Fe, Zn and Cr in the vegetable samples were determined using Buck 210 model Atomic absorption spectrophotometer (AAS) equipped with an air-acetylene burner and hollow cathode lamps. Working standards were also prepared by further dilution of 1000 ppm stock solution of each of the metals and a calibration curve was constructed by plotting absorbance versus concentration. By interpolation, the concentrations of the metals in sample digests were determined. The mean values of six determinations per sample were recorded.

Statistical analysis

All analysis was performed in triplicates. Results were expressed by mean of ± SD. Statistical significance was established using one way analysis of variance (ANOVA). Means were separated according to Duncan's multiple range analysis ($p < 0.05$) using software SPSS 16.0.

RESULTS AND DISCUSSION

The levels of heavy metals (Cu, Cd, Cr, Fe and Zn) in Rosella (*H. sabdariffa*) and Spinach (*A. caudatus*) are as shown in Tables 1 and 2 of Farm A and B. In Rosella (*H. sabdariffa*), obtained from Farm A, the metal levels were: Cu, 30.00 ± 0.15 mg/kg to 31.00 ± 0.18 mg/kg; Fe, 37.39 ± 0.02 mg/kg to 48.67 ± 0.10 mg/kg and Zn, 13.00 ± 0.01 mg/kg to 25.50 ± 0.48 mg/kg. In those obtained from Farm B, the metal levels were: Cu, 31.33 ± 0.25 mg/kg to 33.83 ± 0.03 mg/kg; Fe, 25.06 ± 0.22 mg/kg to 28.47 ± 0.09 mg/kg and Zn, 7.33 ± 0.02 mg/kg. In Spinach (*A. caudatus*) obtained from Farm A, the metal levels were: Cu, 34.33 ± 0.42 mg/kg to 34.50 ± 0.05 mg/kg; Fe, 31.72 ± 0.71 mg/kg to 43.33 ± 0.02 mg/kg and Zn, 10.83 ± 0.17 mg/kg to 21.17 ± 0.14mg/kg. In those obtained from Farm B, the metal levels were: Cu, 35.03 ± 0.50 mg/kg to 38.00 ± 0.10 mg/kg; Fe, 28.47 ± 0.09 mg/kg and Zn, 10.50 ± 0.09 mg/kg to 25.00 ± 0.44 mg/kg. In both Farms A and B chromium and cadmium were not detected. The analysis revealed that Spinach contained higher concentration of copper than Rosella. Although, the maximum values recorded in both the vegetables are within the National Agency for Food and Drug Administration and control's (NAFDAC) maximum tolerable Cu concentration of 40 mg/kg in fresh vegetables. On the other hand, the results

Table 2. Concentration of some heavy metals in Spinach and Rosella from Farm B (mg/kg)

Vegetables/Sampling sites	Cd	Cr	Cu	Fe	Zn
Rosella (*Hibiscus Sabdariffa*)					
Location 1	ND	ND	31.33 ± 0.25	28.47 ± 0.09	7.33 ± 0.02
Location 2	ND	ND	33.83 ± 0.03	25.06 ± 0.22	7.33 ± 0.02
Spinach (*Amaranthus caudatus*)					
Location 1	ND	ND	38.00 ± 0.10	28.47 ± 0.09	25.00 ± 0.44
Location 2	ND	ND	35.03 ± 0.50	28.47 ± 0.09	10.50 ± 0.09
WHO/FAO			20 - 100	10 - 18	3 - 20
NAFDAC			0 - 40	10 - 20	0 - 50

All values represent mean ± standard deviation of triplicate determination. **ND** = Not detected**, WHO** (1996) = World Health organization**, NAFDAC** = National Agency for Food and Drug Administration and control.

were also lower than the published threshold values for mature plant tissue, except Fe with higher level.

The published threshold values are: As, 5 to 10 mg/kg; Fe, 10 to 20 mg/kg; Cu, 20 to 100 mg/kg; Pb, 30 to 300 mg/kg and Zn, 100 to 400 mg/kg (Kabata-Pendias and Pendias, 1984). The critical values or values regarded as excessive are: Zn, >50 – 100 µg/g; Mn, >1000 – 4000 µg/g; Fe, >200 - 500 µg/g; Cu, >7 – 20 µg/g; Pb, >4 – 30 µg/g and Cd, >1 – 3 µg/g; depending on the plants (vegetables) in question (EC–UN/ECE, 1995). The order of the metals contamination in the vegetables was Fe > Cu > Zn in farm A and Cu > Fe > Zn in Farm B.

Statistical test of significance using the Student t-test and ANOVA, showed significant differences ($p < 0.05$) between the levels of the heavy metals in vegetables obtained from the sample sites in Farm A and those from Farm B, with exception of Cu which showed no significant differences ($p > 0.05$). The elevated level of Fe in vegetables in the two gardens could be attributed to excessive usage of fertilizers and other agro-chemicals, as well as the use of waste water in irrigating the soil and of course, the environmental factors in the areas (Uwah et al., 2011). Similarly, the elevated levels of the metals in the vegetables obtained in Farms A and B could be due to possible pollution as a result of the vast agricultural activities going on in the area, and downstream deposition of fertilizers and other agro- chemicals as the Yedzaram River flows into the area. The consumption of these vegetables as food may not pose possible health hazards to human at the time of the study.

Conclusion

Considering the health risk's encountered in diets as a result of high levels of heavy metals in vegetables, the maximum allowable levels of these metals in vegetables should not exceed levels that reflect good agricultural practices. Farmers should be educated on the problems associated with excessive usage of fertilizers and other chemicals, as well as irrigating the crops with waste and all sorts of polluted water and the needs to grow crops with safe levels of heavy metals.

The vegetables contained variable levels of heavy metals (Cd, Cr, Cu, Fe, and Zn), with the exception of those of Fe, the metals levels were lower than the published threshold values considered toxic for mature plant tissue. Similarly, the levels of some of the metals were lower that the established critical limits causing toxicity in plants.

Agronomic practices such as application of fertilizers and use of waste water can affect bioavailability and crop accumulations of heavy metals.

Consumption of these vegetables as food may not constitute possible health hazards to humans at the time of the study.

The results obtained in this study would go a long way in providing a baseline data for the assessment of the distribution of these metals in Spinach (*A. caudatus*) and rosella (*H. sabdariffa*) grown in Uba area in Adamawa State. Further, studies will be carried out on the concentration of the heavy metals in soil of the studied areas.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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