

Original Research Article

Synthesis and Biological Evaluations of Prop-2-enone and Penta-1, 4- dien-3-one Chalcones and their Hydrazone Derivatives

UNDER PEER REVIEW

ABSTRACT

Chalcones are naturally coloured compounds found in plants, characterized by a distinctive chemical structure with two aromatic rings linked by a three-carbon α , β -unsaturated carbonyl bridge. Chalcones synthesis was achieved via modified Claisen-Schmidt condensation utilizing aromatic aldehydes and ketones giving a yield of 80 – 98 % and was then combined with 2, 4-dinitrophenyl hydrazine (2, 4-DNPH) to form hydrazones yielding 58 – 86 %. Products formed were characterized accordingly using $^1\text{H-NMR}$, FTIR, UV, and elemental analysis. The biochemical assay involving antimicrobial activity by employing the agar well diffusion method indicated that the chalcones exhibited no activity against the selected isolates (*E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis*), while the compounds showed good analgesic properties as well as good antioxidant properties as indicated in the DPPH radical scavenging assay with one of the products BE6 showing ($\text{IC}_{50}=11.68 \mu\text{M}$) and the Nitric Oxide bioavailability of BE1A indicating high activity. The total antioxidant determination indicated that the chalcones were better than the hydrazones with BE7 giving a value of $\text{TAC}=8724.02$. This indicates that coupled chalcones may exhibit good analgesic and antioxidant properties but still not a good antimicrobial properties.

Keywords: Antioxidant, DPPH scavenging, Nitric Oxide scavenging, TAC, Analgesic, Antimicrobial.

1. INTRODUCTION

1, 3-diaryl-2-propen-1-one referred to as Chalcones usually exists in the more stable Trans form^[1] however the cis form is also recorded and known to be less stable. This stability and natural abundance make chalcones valuable for drug discovery and pharmaceutical research. Chalcones exhibit antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, making them attractive for further drug development^[2-5] with Clinical examples such as Metochalcone a choleric drug, and Sofalcone an antiulcer agent^[6]. Chalcones also contribute to the sensory qualities of various plants thus influencing their colors, flavors, and aromas^[7].

Scientists continue to explore chalcone derivatives to enhance their bioactivity and selectivity, understanding their structure-activity relationships which is crucial for optimizing these compounds for therapeutic use^[8]. Despite extensive research, the exact mechanisms underlying the biological activities of chalcones remain poorly understood^[9]. However, their versatility and potential across numerous applications make them a focus for continued study.

Hydrazones are an important class of biologically active drug compounds that have attracted interest due to their wide range of pharmacological properties.^[10] Hydrazones are derived from enones, replacing the oxygen atom with a =NNH₂ group, which contributes to their biological diversity.

Hydrazone derivatives have demonstrated notable biological activities, such as anticonvulsant effects^[11] and antidepressant properties^[12]. They also show promise as analgesic agents^[13] and exhibit anti-inflammatory and antiplatelet activities, making them valuable for managing inflammation and preventing clotting^[14]. Hydrazones' antioxidant properties help mitigate oxidative damage^[10]. Their anticancer potential is another area of active investigation. Hydrazones have been explored for other applications, including vasodilation, antiviral activity, and as potential treatments for HIV^[12, 13, 15]

This research aims to synthesize chalcone derivatives that may possess antioxidant, analgesic, and antimicrobial properties and their eventual derivatization by coupling reactions to achieve compounds that will be bioactive.

EXPERIMENTAL SECTION (METHODS)

Evaluation of Compounds

The structure of compounds were confirmed using ¹H-NMR with a JOEL Lambda 400 spectrometer, and FTIR analysis was conducted with a PerkinElmer Frontier (L1050101). Elemental analysis was carried out using an EA3000 CHNSO analyzer. Melting points (uncorrected) were measured using a Gallenkamp melting point apparatus, and compound purity was assessed through thin layer chromatography (TLC) performed using E. Merck Kieselgel 60 F254 plates.

General Method of Chalcone Synthesis

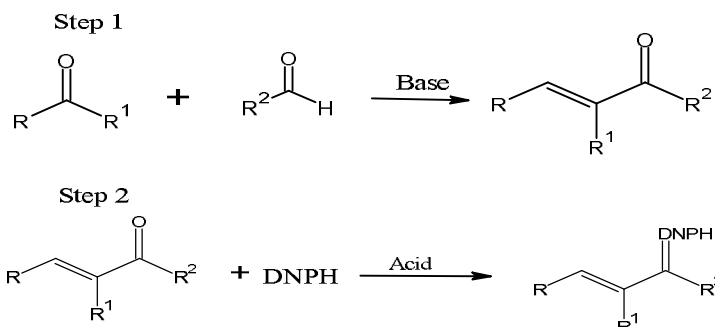
To ice-cold ethanol (20 mL) in a beaker, the desired aldehyde (0.05 mol) and ketone (0.05 mol) were added and stirred using a magnetic stirrer for two hours, maintaining the temperature below 10 °C. Ice-cold 10% potassium hydroxide solution (20 mL) was then added dropwise to the reaction mixture, followed by continued stirring for an additional two hours. The mixture was allowed to stand at room temperature for 72 hours to ensure complete reaction.

Afterward, the reaction mixture was neutralized by adding 50 mL of cold distilled water and 30 mL of 10% acetic acid dropwise with stirring. The resulting crude precipitate was collected by gravity filtration, washed thoroughly with cold water, and air-dried. The crude product was recrystallized from ethanol, dried over silica in a desiccator and then characterized. Seven (7) chalcones (BE1-7) were synthesized.

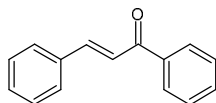
General Method of Hydrazone Synthesis

In a flask equipped with a magnetic stirrer, 2,4-dinitrophenylhydrazine (0.05 mol) was mixed with ethanol (120 mL), followed by the gradual addition of concentrated sulfuric acid (2 mL) with stirring. The appropriate chalcone (0.05 mol) was dissolved in ethanol (60 mL) and added to the 2,4-dinitrophenylhydrazine-ethanol solution under continuous stirring. The mixture was heated under reflux at 70 °C for 15 minutes, and the progress of the reaction was monitored using thin-layer chromatography (TLC).

After completing the reaction, the mixture was allowed to cool to room temperature. The resulting deep orange precipitate was collected by gravity filtration, washed thoroughly, and recrystallized from acetone to enhance purity. The recrystallized hydrazone product was dried in a desiccator and subsequently characterized. Seven (7) hydrazones (BE1A-7) were synthesized.

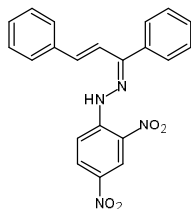


Scheme1: showing the method of chalcone synthesis and their hydrazone counterparts



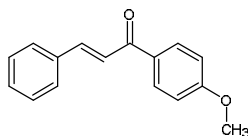
(2E)-1, 3-diphenylprop-2-en-1-one (BE1)

BE1 was synthesized by combining benzaldehyde (5.3 g, 0.05 mol) and acetophenone (6.0g, 0.05 mol). Yield :(9.4g, 95.5%); Color: yellow, mp; 44-47°C, R_f: 0.54; UV (methanol) λ_{max}: 220, 340nm. FTIR: 1662.4 cm⁻¹ (C=O), 1606.5 cm⁻¹ (C=C), 3056.4 cm⁻¹ (C-H¹H NMR (400 MHz, Chloroform-d) δ 8.05 – 8.00 (m, 2H), 7.82 (d, J = 15.8 Hz, 1H), 7.65 (dtd, J = 5.8, 3.5, 1.6 Hz, 2H), 7.63 – 7.55 (m, 2H), 7.55 – 7.49 (m, 2H), 7.47 – 7.39 (m, 3H). ¹³C NMR (125 MHz, Chloroform-d) δ 190.61, 144.88, 138.24, 134.91, 132.81, 130.57, 128.99, 128.65, 128.53, 128.47, 122.13. HRMS (ESI): C₁₅H₁₃O [M+H]⁺ calculated for: 209.0961, found: 209.0959.



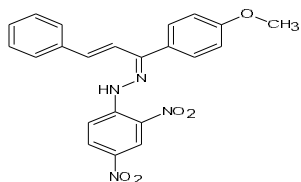
1-(2, 4-dinitrophenyl)-2-[(2E)-1, 3-diphenylprop-2-en-1-ylidene] hydrazine (BE1A)

BE1A was synthesized by combining 2, 4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE1 (10.4 g, 0.05 mol). Yield :(16.736g, 85.5 %); Color: deep orange, mp; 120-123°C, R_f: 0.54; UV (methanol) λ_{max}: 280,320,460nm, FTIR: 1587.8 cm⁻¹ (C=N), 3369.5 cm⁻¹ (N-H), 1416.4 cm⁻¹ (C-H), 1110 cm⁻¹ (N-O). ¹H NMR (400 MHz, DMSO-d₆) δ 12.93 – 12.81 (m, 1H), 12.65 – 12.38 (m, 3H), 12.38 – 12.10 (m, 5H), 8.10 (s, 2H), 7.26 (p, J = 1.8 Hz, 10H).



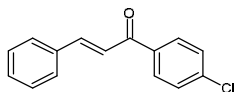
(2E)-1-(4-methoxyphenyl)-3-phenylprop-2-en-1-one (BE2)

BE2 was synthesized by combining 4-methoxy benzaldehyde (5.0 g, 0.04 mol) and acetophenone (6.0 g, 0.05 mol). Yield : (8.7g, 98.6%); Color: Yellow; mp; 85-87°C, R_f: 0.6; UV (methanol) λ_{max}: 240,280, 340 nm FTIR (cm⁻¹): 1654.9 (C=O), 1572.9 (C=C) ¹H NMR (400 MHz, Chloroform-*d*) δ 8.03 – 7.99 (m, 2H), 7.79 (d, *J* = 15.7 Hz, 1H), 7.64 – 7.55 (m, 3H), 7.54 – 7.47 (m, 2H), 7.42 (d, *J* = 15.7 Hz, 1H), 7.02 – 6.90 (m, 2H), 3.86 (s, 3H). ¹³C NMR (125 MHz, Chloroform-*d*) δ 190.65, 161.71, 144.74, 138.54, 132.57, 130.25, 128.58, 128.44, 127.65, 119.83, 114.45, 55.44. **HRMS (ESI)**: C₁₆H₁₄O₂ [M+H]⁺ calculated for: 239.1067, found: 239.1063.



1-(2, 4-dinitrophenyl)-2-[(2E)-1-(4-methoxyphenyl)-3-phenylprop-2-en-1-ylidene]hydrazine (BE2A)

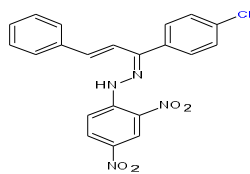
BE2A was synthesized by combining 2, 4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE2 (11.9 g, 0.05 mol). Yield : (17.3g, 82.7%); Color: red precipitate; mp; 145-148°C, R_f: 0.7; UV (methanol) λ_{max}: 320, 360,440nm FTIR (cm⁻¹): 1580.4 (C=N), 3287.5 (N-H), 1509.9 (C-H), 1133.1 (N-O). ¹H NMR (400 MHz, DMSO-*d*6) δ 11.59 (s, 1H), 8.90 (d, *J* = 2.7 Hz, 1H), 8.45 (ddd, *J* = 12.3, 9.5, 2.6 Hz, 2H), 8.08 (dd, *J* = 9.6, 2.2 Hz, 2H), 7.81 (dd, *J* = 6.8, 3.0 Hz, 3H), 7.74 – 7.67 (m, 4H),



(2E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (BE3)

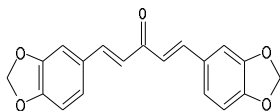
BE3 was synthesized by combining 4-chlorobenzaldehyde (5.0 g, 0.04 mol) and acetophenone (6.0 g, 0.05 mol). Yield : (10.6 g, 92.72%); Color: Cream; mp; 60-62°C, R_f: 0.5; UV (methanol) λ_{max}: 240,320 nm FTIR (cm⁻¹): 1654.9 (C=O), 1591.6 (C=C), 715.6 (C-Cl) ¹H NMR (400 MHz, Chloroform-*d*) δ 8.05 – 7.95 (m, 2H), 7.76 (d, *J* = 15.7 Hz, 1H), 7.63 – 7.55 (m, 3H), 7.52 (dd, *J* = 6.0, 2.0 Hz, 3H), 7.42 – 7.37 (m,

2H). ^{13}C NMR (125 MHz, Chloroform-*d*) δ 190.27, 143.34, 138.05, 136.46, 133.40, 132.95, 129.61, 129.28, 128.70, 128.52, 122.50. HRMS (ESI): $\text{C}_{15}\text{H}_{12}\text{ClO}$ $[\text{M}+\text{H}]^+$ calculated for: 243.0571, found: 243.0569.



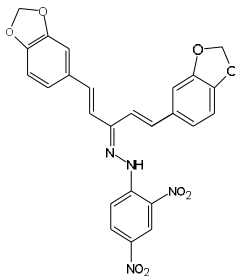
1-[(2E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-ylidene]-2-(2,4-dinitrophenyl)hydrazine (BE3A)

BE3A was synthesized by combining 2,4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE3 (12.1 g, 0.05 mol). Yield: (16.6 g, 77.9%); Color: deep red precipitate; mp 179-181°C, R_f : 0.5; UV (methanol) λ_{max} : 360, 460nm, FTIR (cm^{-1}): 1610.2 (C=N), 3298.7 (N-H), 1487.2 (C-H), 1286.0 (N-O). ^1H NMR (400 MHz, DMSO-*d*6) δ 11.59 (s, 1H), 8.90 (s, 1H), 8.47 (dd, J = 19.4, 9.6 Hz, 1H), 8.10 (dd, J = 9.6, 6.9 Hz, 1H), 7.87 – 7.76 (m, 4H), 7.75 – 7.62 (m, 1H), 7.57 (s, 1H), 7.57 – 7.40 (m, 6H), 7.22 (d, J = 16.8 Hz, 1H).



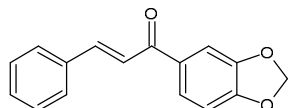
(1E, 4E)-1,5-bis(2H-1,3-benzodioxol-5-yl)penta-1,4-dien-3-one (BE4)

BE4 was synthesized by combining Piperonal (7.5 g, 0.05 mol) and acetone (5.8 g, 0.05 mol). Yield: (6.9g, 90.86%); Color: Yellow; mp: 92-94°C, R_f : 0.4; UV (methanol) λ_{max} : 280, 340 nm FTIR (cm^{-1}): 1669.8 (C=O), 1640.0 (C=C) ^1H NMR (400 MHz, Chloroform-*d*) δ 7.19 – 6.98 (m, 1H), 6.83 (t, J = 6.9 Hz, 1H), 6.02 (d, J = 3.3 Hz, 1H), 2.35 (s, 1H).



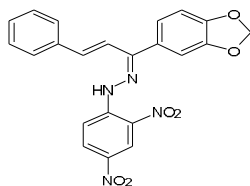
1-[(1E, 4E)-1,5-bis(2H-1,3-benzodioxol-5-yl)penta-1,4-dien-3-ylidene]-2-(2,4-dinitrophenyl)hydrazine (BE4A)

BE4A was synthesized by combining 2, 4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE4 (16.1 g, 0.05 mol). Yield :(17.83g, 70.1%); Color: red; mp; 172-174°C, R_f: 0.68; UV (methanol) λ_{max}: 300, 480 nm FTIR (cm⁻¹): 1610.2 (C=N), 3309.9 (N-H), 1252.4 (N-O). ¹H NMR (400 MHz, DMSO-d₆) δ 8.90 (d, J = 2.6 Hz, 2H), 8.87 (s, 1H), 8.44 (dd, J = 9.5, 2.7 Hz, 2H), 8.04 – 7.94 (m, 3H), 7.44 (d, J = 16.5 Hz, 2H), 7.35 (d, J = 1.6 Hz, 2H), 7.32 – 7.23 (m, 2H),



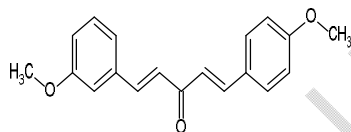
(2E)-1-(2H-1, 3-benzodioxol-5-yl)-3-phenylprop-2-en-1-one (BE5)

BE5 was synthesized by combining Piperonal (7.5 g, 0.05 mol) and acetophenone (6.0 g, 0.05 mol). Yield :(9.5 g, 79.8%); Color: Yellow; mp; 78-81°C, R_f: 0.6; UV (methanol) λ_{max}: 260, 300, 340 nm FTIR (cm⁻¹): 1654.9 (C=O), 1587.8 (C=C). ¹H NMR (400 MHz, Chloroform-d) δ 8.07 – 7.97 (m, 2H), 7.74 (d, J = 15.6 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.53 – 7.47 (m, 2H), 7.37 (d, J = 15.6 Hz, 1H), 7.21 – 7.10 (m, 2H), 6.85 (d, J = 8.0 Hz, 1H), 6.03 (s, 2H). ¹³C NMR (125 MHz, Chloroform-d) δ 190.45, 149.94, 148.44, 144.71, 138.42, 132.66, 129.39, 128.61, 128.43, 125.26, 120.14, 108.70, 106.67, 101.66. **HRMS (ESI):** C₁₆H₁₃O₃ [M+H]⁺ calculated for: 253.0859, found: 253.0855.



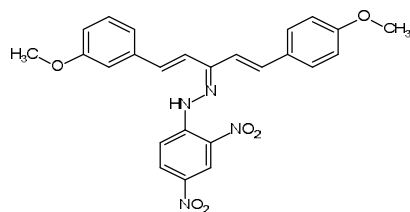
1-(2, 4-dinitrophenyl)-2-[(2E)-1-(4-methoxyphenyl)-3-phenylprop-2-en-1-ylidene] hydrazine (BE5A)

BE5A was synthesized by combining 2, 4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE5 (12.6 g, 0.05 mol). Yield: (12.47g, 73.6%), Color: red precipitate, mp; 150-154°C, R_f: 0.7; UV (methanol) λ_{max}: 320, 380, 440nm, FTIR (cm⁻¹): 1610.2 (C=N), 3295.0 (N-H), 1487.2 (R-Ar), 1448.2 (C-H), 1125.5 (N-O). ¹H NMR (400 MHz, DMSO-d₆) δ 8.96 – 6.69 (m, 1H).



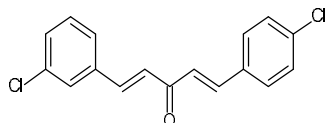
(1E, 4E)-1-(3-methoxyphenyl)-5-(4-methoxyphenyl) penta-1, 4-dien-3-one (BE6)

BE6 was synthesized by mixing 4-methoxy benzaldehyde (6.8 g, 0.05 mol) and acetone (5.8 g, 0.05 mol). Yield : (5.5 g, 79.8%); Color: Cream; mp; 75-78°C, R_f: 0.7; UV (methanol) λ_{max}: 300, 340 nm FTIR: 1654.4 (C=O), 1595.3 (C=C). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.70 (d, J = 15.9 Hz, 1H), 7.61 – 7.43 (m, 5H), 7.00 – 6.87 (m, 5H), 6.61 (d, J = 16.2 Hz, 1H), 3.85 (d, J = 3.7 Hz, 6H). ¹³C NMR (125 MHz, Chloroform-*d*) δ 198.44, 188.88, 161.63, 161.56, 143.27, 142.70, 130.09, 129.98, 127.65, 127.08, 125.06, 123.51, 114.46, 114.43, 55.42, 27.42. **HRMS (ESI):** C₁₉H₁₉O₃ [M+H]⁺ calculated for: 295.1329, found: 295.1325.



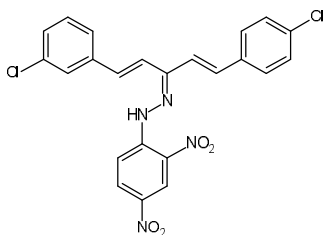
1-[(1E, 4E)-1-(3-methoxyphenyl)-5-(4-methoxyphenyl) penta-1, 4-dien-3-ylidene]-2-(2, 4-dinitrophenyl) hydrazine (BE6A)

BE6A was synthesized by combining 2, 4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE6 (14.7 g, 0.05 mol). Yield : (15.5 g, 65.4%); Color: Deep red precipitate; mp 149-151°C, R_f: 0.7; UV (methanol) λ_{max}: 300, 360, 480 nm, FTIR (cm⁻¹): 1587.8 (C=N), 3302.4 (N-H), 1487.2 (R-Ar), 1448.2 (C-H), 1224.9 (N-O). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.73 (s, 1H), 8.05 (dd, J = 9.7, 2.7 Hz, 1H), 7.60 (dt, J = 9.6, 2.4 Hz, 1H), 7.17 (dd, J = 22.0, 9.6 Hz, 1H), 6.91 – 6.74 (m, 4H), 6.52 – 6.37 (m, 2H), 6.24 (dd, J = 16.2, 8.5 Hz, 3H), 6.17 – 6.07 (m, 3H), 2.98 (d, J = 11.3 Hz, 6H), 1.67 (p, J = 1.9 Hz, 13H), 1.47 (s, 1H), -0.83 (s, 1H).



(1E, 4E)-1-(3-chlorophenyl)-5-(4-chlorophenyl) penta-1, 4-dien-3-one (BE7)

BE7 was synthesized by combining 4-chlorobenzaldehyde (5.0 g, 0.04 mol) and acetophenone (6.0 g, 0.05 mol). Yield : (6.9 g, 95.6%); Color: Yellow; mp; 58-60°C, R_f: 0.4; UV (methanol) λ_{max}: 260, 320 nm FTIR (cm⁻¹): 1647.5 (C=O) 1587.8 (C=C), 823.7 (C-Cl). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.07 – 8.00 (m, 2H), 7.81 (d, J = 15.7 Hz, 1H), 7.64 (d, J = 6.5 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.57 – 7.48 (m, 2H), 7.44 (d, J = 15.6 Hz, 1H), 7.01 – 6.93 (m, 2H), 3.88 (s, 3H).



1-[(1E, 4E)-1-(3-chlorophenyl)-5-(4-chlorophenyl) penta-1, 4-dien-3-ylidene]-2-(2, 4-dinitrophenyl) hydrazine (BE7A)

BE7A was synthesized by combining 2, 4-dinitrophenylhydrazine (9.9 g, 0.05 mol) and BE7 (15.16 g, 0.05 mol). Yield : (13.8g, 57.1%); Color: red precipitate; mp 150-152°C, R_f: 0.6; UV (methanol) λ_{max}: 360, 460nm, FTIR: 1613.9 (C=N), 3306.1 (N-H), 1448.2 (C-H), 1293.4 (N-O). ¹H NMR (400 MHz, DMSO-d₆) δ 1.70 – 1.64 (m, 11H), -0.83 (s, 1H).

Antioxidant Activity

1, 1-Diphenyl-2 Picrylhydrazyl (DPPH) Radical Scavenging

Evaluation of the scavenging potential of the synthesized compounds against DPPH free radicals was carried out following the procedure proposed by Gyamfi *et al.*, (1999) ^[16]. In this method, suitable dilutions of the samples dissolved in 1 ml DMSO were mixed with 1 ml of a 0.4 mMethanolic mixture containing DPPH radicals. The solution was then kept in the dark for 30 minutes, after which its absorbance was measured at 516 nm using a UV-visible spectrophotometer. The DPPH radical scavenging potential was subsequently calculated.

A linear regression analysis was conducted using the concentration (log C) versus radical scavenging capability (%) plot to determine the IC₅₀ values of the test sample. GraphPad Prism version 10.0 was used for the IC₅₀ determination.

Nitric Oxide Radical Scavenging Assay

The Greiss reaction was employed to quantify nitric oxide (NO) generated by sodium nitroprusside. A solution of sodium nitroprusside (5 mM) in phosphate buffer of pH 7.3 was combined with various concentrations of the products dissolved in DMSO and incubated at 25 °C for 3 hours. Subsequently, the samples were reacted with a Greiss reagent. The absorbance of the samples was measured at 546 nm against a blank solution using a UV-visible spectrophotometer. The test was performed in triplicate, and the IC₅₀ values calculated.

Griggs Reagent

This was formulated by dissolving 1 g of sulphanilamide, 5 mL of phosphoric acid, and 0.1 g of 1-naphtylenediamine dihydrochloride in 100 mL of distilled water.

Total Antioxidant by the Phosphomolybdenum Method

Exactly 1 mL comprising 0.6 M sulfuric acid, sodium phosphate (28 mM), and 4 mM ammonium molybdate was combined with 0.1 mL of the sample solution (corresponding to 100 mg). Instead of the sample, ethanol (0.1 mL) was utilized as the blank. The tubes were firmly sealed and incubated for 90 minutes in a boiling water bath at 95°C. The absorbance of each aqueous solution was measured at 695 nm after the samples were cooled to room temperature. The antioxidant capacity was expressed in milligrams of Gallic acid.

Analgesic Activity

Preparation of Red Blood Cell Suspension

A blood sample (5 mL) was collected from a healthy volunteer who had abstained from NSAIDs for two weeks before the experiment and transferred to a centrifuge tube. The sample was centrifuged at 3000 rpm for 10 minutes, then washed three times with an equal volume of normal saline. Finally, it was adjusted to a volume of 100 mL by adding 90% normal saline.

Preparation of Hyposaline

Disodium hydrogen phosphate (1.38 g) was weighed into a beaker using an analytical weighing balance it was dissolved in distilled water and made up to 100 mL. Exactly 2.68 g of sodium dihydrogen phosphate was weighed and dissolved in distilled water and made up to 100 mL. Disodium hydrogen phosphate (28 mL) was measured using a measuring cylinder and transferred into a 100-volumetric glass. Sodium dihydrogen phosphate (72 mL) was measured and transferred into the same volumetric glass and was mixed appropriately (PBC is formed). Exactly 0.9 g of NaCl is weighed and dissolved with PBC and made up to 100 mL.

Preparation of Stock Solution with Sample and Standard

Exactly 0.025 g of the chalcones and hydrazones derivative was weighed using an analytical weighing balance and dissolved with dimethyl sulfur oxide (DMSO). It was made up to 25 mL with DMSO. The same procedure was done for the rest chalcones, hydrazones, and diclofenac the reference standard.

Hypotonic solution-induced hemolysis

The control was prepared by mixing 2ml of hyposaline solution and 10 μ L of red blood cells (RBC) in a test tube. 0.1 mL of the samples, 1.9 mL of hyposaline, and 10 μ L of the RBC solution were pipetted into a test tube using the micropipette and mixed properly. The procedure was done in triplicate and allowed to sit for 10 minutes before being subjected to a UV-visible spectrophotometer set to a wavelength of 540 nm. The readings were carefully recorded, and the same procedure was done for all the chalcone and hydrazone derivatives stock at different concentrations (200 μ g/mL, 400 μ g/mL, 600 μ g/mL, 800 μ g/mL, and 1000 μ g/mL).

Antimicrobial Activity

Preparation of Mueller Hinton Agar

Mueller Hinton agar (8.3 g) was weighed and dissolved in distilled water (485 mL) and autoclaved at a temperature of 121° C for 15 minutes. Then the agar solution was allowed to cool, poured into sixteen (16) petri-dish plates, allowed to solidify, and then dried in the dryer until the petri-dish plates were completely dried and free of moisture.

Preparation of standard and synthesized solution

McCartney bottles (16) were autoclaved at a temperature of 121°C for 15 minutes. Exactly 0.2 g of chalcones were weighed into a McCartney bottle and dissolved in 8 ml of DMSO. Exactly 4ml of DMSO was pipetted to three of the McCartney bottles each and labeled appropriately. The serial dilution process was carried out to achieve a concentration of 3125 μ g/ml after which 4 ml of the solution was pipetted and discharged. Ciprofloxacin solution infusion containing 200 mg per 100 mL was used to prepare the standard of 10 μ g/mL. 100ml of distilled water was weighed and transferred to a syrup bottle, it was then autoclaved at 121°C for 15 minutes and allowed to cool. Exactly 0.5 mL of water was discharged, and ciprofloxacin solution (0.5ml) was then pipetted inside and shaken properly.

Procedure

Two Gram-positive clinical isolates of *Bacillus subtilis* and *Staphylococcus aureus* were used, also two gram negative clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* were used. The Petri dishes were labeled according to the isolates and a sterile swap stick was used to dip inside an already cultured broth medium of the isolates and was used to swap around the petri-dish plates and allowed to dry. Six holes were bored using a cork borer of 7mm diameter of which the cork borer was sterilized under the flame after each hole was drilled. Molten agar was used to seal each hole with the aid of a Pasteur pipette and allowed to solidify. The holes were labeled appropriately and placed in their respective labeled holes and then incubated for 24 hours and their respective zone of incubation was measured. This was done for all concentrations of 12,500 $\mu\text{g/mL}$, 6250 $\mu\text{g/mL}$, and 3125 $\mu\text{g/mL}$ and also for all isolates. This procedure was done in duplicate.

3. RESULTS AND DISCUSSION

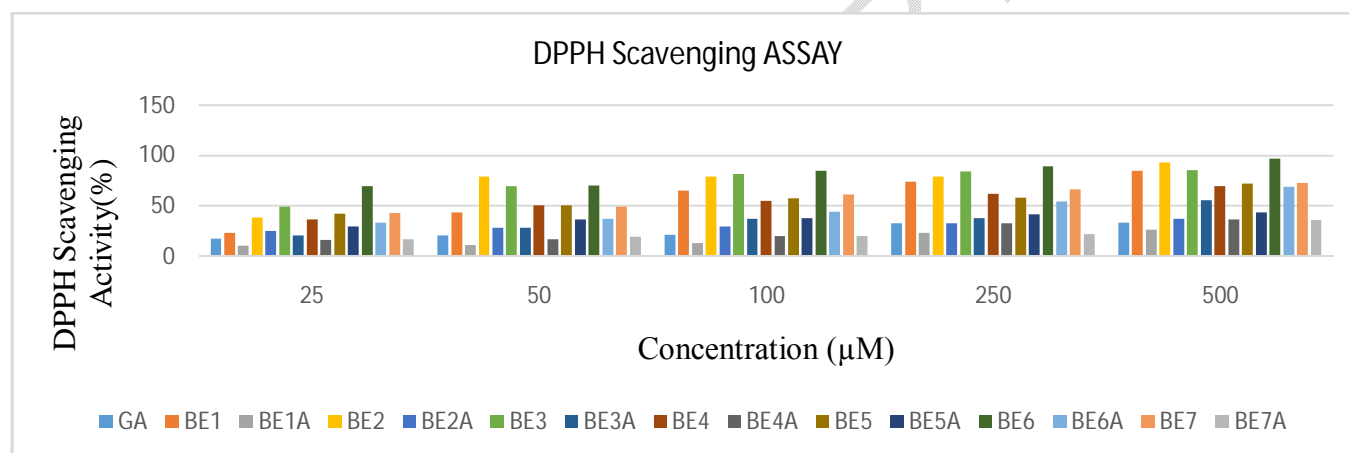


Fig 1: DPPH Radical scavenging activities of synthesized compounds

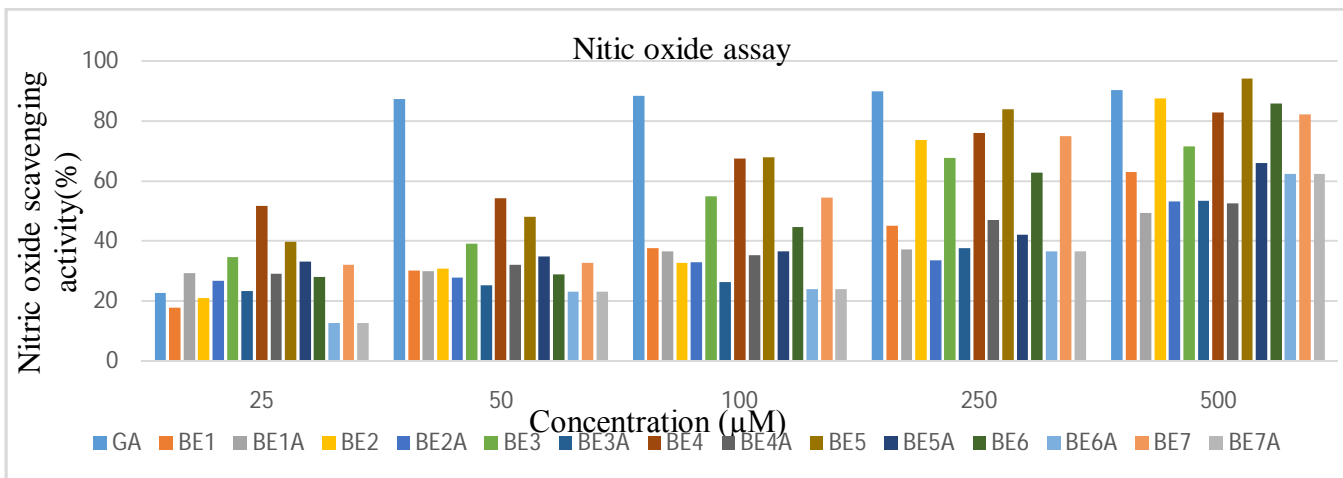


Fig 2: Nitric oxide radical scavenging activities of synthesized compounds

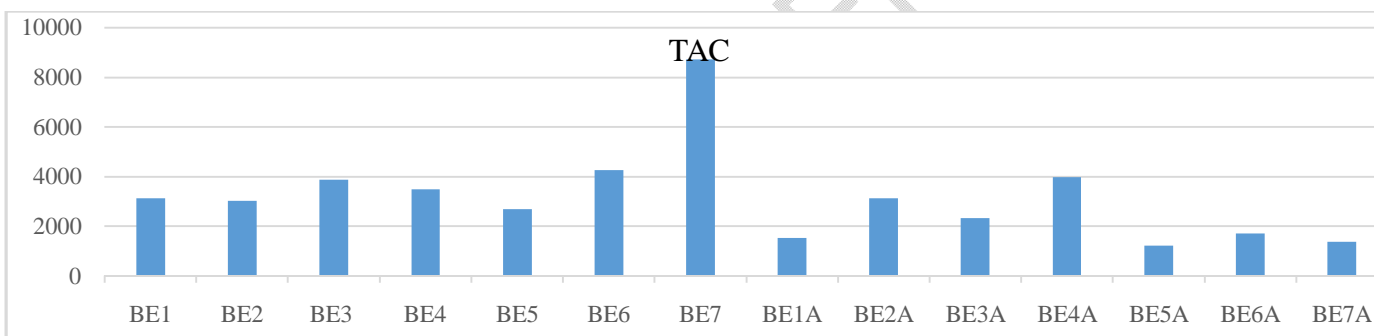


Fig 3: Total antioxidant capacity of synthesized compounds

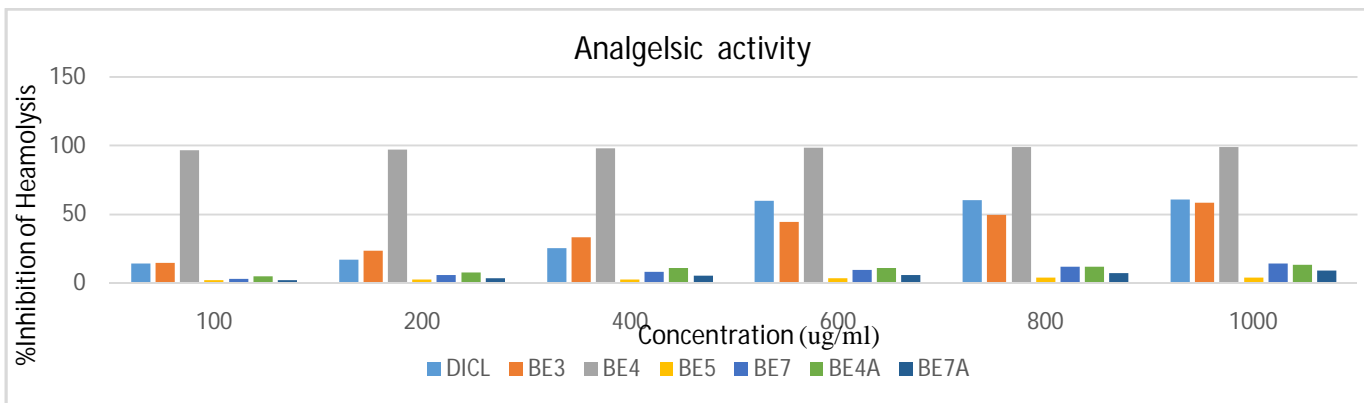


Fig 4: Analgesic activity of synthesized compounds.

Table 1: Anti-microbial screening of synthesized sample against bacterial isolates zone of inhibition

Sample	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
B ₁	-	-	-	-
B ₂	-	-	-	-
BE3 ₁	-	-	-	-

BE3 ₂	-	-	-	-
BE4 ₁	-	-	-	-
BE4 ₂	-	-	-	-
BE7 ₁	-	-	-	-
BE7 ₂	-	-	-	-
BE4A ₁	-	-	-	-
BE4A ₂	-	-	-	-
BE7A ₁	-	-	-	-
BE7A ₂	-	-	-	-

-Represent no activity

Table 2: IC₅₀ values for synthesized chalcones

Compounds	IC ₅₀ values (DPPH)	IC ₅₀ values (NO)	IC ₅₀ values (Analgesic)
BE1	58.31	76.48	NA
BE2	22.76	216.4	NA
BE3	18.0	39.7	129.0
BE4	22.34	19.53	2.976

BE5	17.06	49.13	186.6
BE6	11.68	145.0	NA
BE7	20.58	75.72	131.0
Ref. Standard	GA =34.26	GA=28.31	DICL=154.7

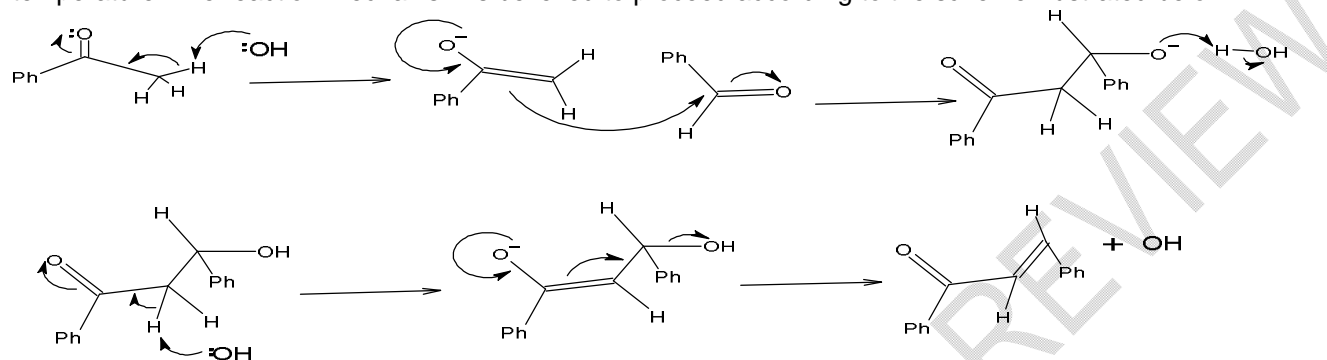
Table 3: IC₅₀ values for synthesized Hydrazones

Compounds	IC ₅₀ values (DPPH)	IC ₅₀ values (NO)	IC ₅₀ values (Analgesic)
BE1A	109.2	19.06	NA
BE2A	12.41	30.60	NA
BE3A	49.86	79.31	NA
BE4A	77.01	29.05	355.1
BE5A	11.97	33.85	NA
BE6	39.64	38.45	NA
BE7	39.03	498.3	122
Ref. Standard	GA =34.26	GA=28.31	DICL=154.7

Synthesis

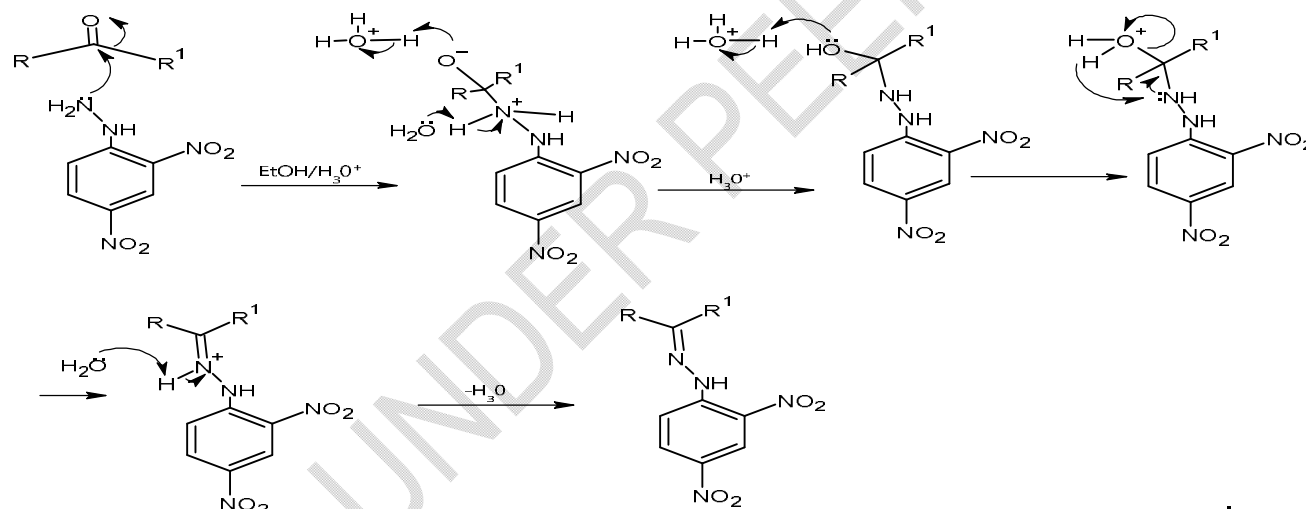
Many conventional medicines and some under clinical investigation were obtained via chemical synthesis and computer-aided designs using various methods^[17-21]. A series of chalcone derivatives were synthesized through the utilization of the Claisen-Schmidt condensation

reaction, which involves the reaction of aromatic aldehydes with suitable aromatic ketones in the presence of aqueous alkali at a low temperature. The reaction mechanism is believed to proceed according to the scheme illustrated below.



Scheme 2: Chalcones Synthesis Mechanism

Subsequently, to form the hydrazones from the chalcones, the coupling reaction with chalcones and 2, 4-dinitrophenylhydrazine (2, 4-DNPH) where achieved in ethanol catalyzed by sulphuric acid. The perceived reaction mechanism is likely as shown in the scheme below.



Scheme 3: Coupling Mechanism of Hydrazone

The synthesized chalcones all have high percentage yield above 85.5% except **BE5** and **BE6** which has slightly lower percentage yield (79.8% and 85.5%). Similarly, the hydrazones generated from the coupling reaction with 2, 4-dinitrophenyl hydrazine (DNPH), gave lower yields (65.4% and 57.1% respectively). The plausible reason responsible for this low yield may just be steric.

All the chalcone compounds exhibit characteristic absorptions in the UV-visible region. Specifically, the chalcones show absorption peaks at wavelengths such as 220 nm, 240 nm, 280 nm, and 340 nm. Upon coupling to form hydrazone derivatives, these compounds demonstrate absorptions at 260 nm, 280 nm, 320 nm, 360 nm, 440 nm, and 480 nm. The bathochromic shifts observed between the chalcones and their hydrazones are attributed to extended conjugation following the coupling process, confirming the successful formation of the hydrazone derivatives.

In the FTIR spectra, all chalcones exhibit a distinct C=O stretch between 1654.9 cm^{-1} and 1669.8 cm^{-1} . After coupling to form the hydrazone derivatives, the C=O stretch disappears, replaced by a characteristic C=N stretch around 1580 cm^{-1} to 1610 cm^{-1} . Additionally, the presence of N-H and N-O vibrational signals further supports the formation of hydrazones, indicating the effectiveness of the coupling process.

The characterized chalcones exhibit proton ^1NMR features consistent with their conjugated aromatic systems and α,β -unsaturated carbonyl groups, with aromatic protons resonating between δ 7.25 and δ 8.03 ppm, including singlets from δ 7.57 to δ 7.80 ppm, doublets at δ 7.79 and δ 8.03 ppm, and multiplets from δ 7.57 to δ 7.77 ppm, reflecting conjugation effects. The synthesized hydrazones display distinct signals confirming the hydrazone functional group (-C=N-NH), with a prominent singlet at δ 11.59 ppm for the NH proton, doublets between δ 8.45 and δ 8.90 ppm, and multiplets from δ 7.57 to δ 7.87 ppm, indicating changes in electron density while preserving the aromatic system. Overall, the NMR data affirm the successful synthesis of hydrazones from chalcones, as evidenced by the NH peak around δ 11.59 ppm and significant shifts in the aromatic region, confirming the structural transformations and retention of the conjugated aromatic integrity alongside the new functionality.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity gauges a substance's capacity to neutralize or diminish DPPH radicals. Commonly used to assess the antioxidant potential of compounds, the assay relies on lower IC_{50} values indicating greater efficiency in neutralizing free radicals. Substances demonstrating significant DPPH radical scavenging activity are deemed valuable in addressing oxidative stress and reducing the risk of various diseases linked to free radical damage.

The chalcone derivatives can be ranked in terms of scavenging DPPH radical activity from highest to lowest as follows: **BE6 > BE5 > BE3 > BE7 > BE4 > BE2 > BE1**. All synthesized chalcones apart from **BE1** possess IC_{50} ($\text{IC}_{50} = 58.3\ \mu\text{M}$) higher than Gallic acid ($\text{IC}_{50} = 34.26\ \mu\text{M}$) the reference standard indicating that all the synthesized chalcones are potent scavengers of DPPH radicals.

Although **BE1** has the least steric hindrance, the decrease in its DPPH radical scavenging potential might be attributed to the absence of substituents on both its ketonic and aldehydic portions. From the experimental data, it was observed that the presence of substituents in either the ketonic or aldehydic portion tends to enhance the DPPH radical scavenging potential.

Structural activity relationship based on DPPH radical scavenging activity

From the experimental data, a structural activity relationship (SAR) for the DPPH radical scavenging potential was observed. It was observed that the chalcone compound with no substituents in both the A and B ring has the least potency to scavenge the DPPH radical. The methoxy (-OCH₃) substituent in both ring A and B gives chalcones with higher DPPH scavenging potential while a methoxy group in only ring A yields a less potent DPPH scavenging potential. A diether substituent in ring A also yields chalcones with high DPPH radical scavenging ability. The presence of the chloro group in ring A also yields chalcones with good DPPH radical scavenging ability. Chlorine substituent in both ring A and ring B gives chalcones with good DPPH radical scavenging potentials but are slightly less potent than chalcones having chlorine in only their A ring, and chalcones with a di-ether substituent in both A and B ring also displays good DPPH scavenging potential but are slightly inferior to chalcones having only di-ether substituent in the ring A only.

Among the 2,4-DNPH coupled products, **BE5A** displayed the highest DPPH scavenging activity, as indicated by its significantly lesser IC₅₀ value of 11.94 μM surpassing Gallic acid (IC₅₀=34.26), the reference standard. This increased potency can be attributed to the presence of an ether group in the A ring. On the other hand, **BE1A** exhibited the lowest DPPH scavenging activity among the coupled products, with an IC₅₀ value of 109.2 μM. This higher IC₅₀ value might be due to the absence of substituents in either the ketonic or aldehydic portions of the compound.

The 2,4-DNPH coupled products can be ranked in terms of DPPH radical scavenging activity from highest to lowest as follows: **BE5A>BE2A>BE7A>BE6A>BE3A>BE4A>BE1A**.

Additionally, **BE5A** (IC₅₀=11.9), and **BE2A** (IC₅₀=12.41) exhibited lesser IC₅₀ values than Gallic acid (IC₅₀=34.2), suggesting that these coupled products have a better ability to neutralize the DPPH radicals compared to the reference standard antioxidant. In contrast, **BE1A** (IC₅₀=109.2), **BE3A** (IC₅₀=49.86), and **BE4A** (IC₅₀=77.01) exhibited IC₅₀ values much higher than that of Gallic acid, indicating relatively weaker antioxidant activity compared to the reference standard. While **BE6A** (IC₅₀=39.64) and **BE7A** (IC₅₀=39.03) have similar IC₅₀ values as that of the reference standard Gallic acid.

The comparison of chalcone derivatives with their respective coupled products shows that the antioxidant activity can vary as some chalcone derivatives (**BE1**, **BE3**, **BE4**, **BE6**, and **BE7**) exhibit stronger antioxidant activity than their coupled counterparts (**BE1A**, **BE3A**, **BE4A**, **BE6A**, and **BE7A**). However, **BE2A** and **BE6A** display stronger antioxidant activity than their parent chalcone **BE2** and **BE6**. In general, the hydrazones showed higher IC₅₀ values than their parent chalcone, therefore they are less potent scavengers of DPPH radicals. This could be attributed to steric hindrance as the hydrazones have a bulkier structure than their parent chalcones. Significant resemblances were noted when compared with previous research findings which reported the potent DPPH radical scavenging activities of chalcones and their derivatives.^[10, 22, 23]

Total Antioxidant Capacity (TAC)

The TAC measurement provides valuable information about the presence and concentration of antioxidants in compounds by evaluating the ability of the synthesized compounds to reduce molybdenum (IV) to molybdenum (V). Among the chalcones, (1E, 4E)-1-(3-chlorophenyl)-5-(4-chlorophenyl) penta-1, 4-dien-3-one (**BE7**) exhibits the highest total antioxidant capacity with a TAC value of 8724.02. This suggests that **BE7** has the most potential to scavenge free radicals and can serve as a robust protector against oxidative damage. This observed phenomenon can be attributed presence of -Cl substituents at *para* positions in both the ketonic and aldehydic portions of the compound.

Following **BE7**, **BE6** (TAC value = 4277.597) demonstrates the second-highest total antioxidant capacity, this might be due to the presence of a methoxy group in both the ketonic and aldehydic portions of the compound. Based on the observed phenomenon, it is reasonable to conclude that the inclusion of substituents with electron-withdrawing ability such as -Cl and OCH₃ groups at the para position in both the ketonic and aldehydic segments of the chalcones tends to enhance their overall antioxidant capacity. **BE3** and **BE4** also display considerable total antioxidant capacity, with TAC values of 3874.459 and 3483.709 respectively, indicating their ability to effectively neutralize free radicals. On the other hand, **BE1**, **BE2**, and **BE5** show relatively lower total antioxidant capacity with TAC values of 3132.716, 3039.216, and 2704.082 respectively, suggesting a comparatively weaker ability to scavenge free radicals and provide antioxidant defense. This could be attributed to the fact that **BE1**, **BE2**, and **BE5** have substituents in only their ketonic segments or no substituents at all.

Structural activity relationship based on total antioxidant capacity

From the experimental data, a structural activity relationship (SAR) for the total antioxidant capacity was observed. It was noted that the chalcone compound with no substituents in both the A and B rings has the least total antioxidant capacity. The methoxy (-OCH₃) substituent in both rings A and B gives chalcones with higher total antioxidant capacity while a methoxy substituent in only ring A gives a reduced total antioxidant capacity. Chlorine substituent in both ring A and B gives chalcones with higher total antioxidant capacity while a chlorine substituent in only ring A gives a reduced total antioxidant capacity.

Among the coupled products, **BE4A** (TAC value = 3989.704) exhibits the highest total antioxidant potential, indicating a significant concentration of antioxidant compounds. This suggests that **BE4A** has the potential to be a potent scavenger of free radicals and a robust protector against oxidative damage. The reason for this behavior might be due to the presence of an ether group in both the ketonic and the aldehydic portion of the compound.

Following **BE4A**, **BE2A** demonstrates the second-highest total antioxidant capacity with a TAC value of 3137.715, highlighting its notable antioxidant potential. **BE3A** (TAC= 2330.651) and **BE6A** (TAC= 1721.509) also display considerable total antioxidant capacity, indicating their ability to effectively neutralize free radicals and provide antioxidant defense. On the other hand, **BE7A**, **BE1A**, and **BE5A** show relatively lower total antioxidant capacity, suggesting a comparatively weaker ability to scavenge free radicals and provide antioxidant defense.

In comparing the TAC of the chalcone derivatives and their respective coupled products, it is evident that the chalcone derivatives generally exhibit higher total antioxidant capacity values. This suggests that the coupling process with 2, 4-DNPH leads to a reduction in the overall antioxidant potential of the compounds. Significant parallels were noted in the total antioxidant capacity exhibited by both chalcones and their hydrazone derivatives in comparison to previous research findings which have documented the strong total antioxidant capacity of chalcones ^[24, 25].

Nitric oxide scavenging activity

Assessing the scavenging ability of nitric oxide is essential for comprehending the potential health advantages of a substance, especially in the realm of anti-inflammatory and antioxidant properties. Nitric oxide (NO) scavenging activity pertains to a substance's capability to neutralize or hinder the actions of nitric oxide, a signaling molecule implicated in diverse physiological processes within the body. Among

the chalcone derivatives and Gallic acid (GA) used as a reference standard in the nitric oxide scavenging assay, **BE4** showed the lowest IC_{50} value of 19.53 μ M, indicating its superior ability to scavenge nitric oxide radicals compared to **GA**, with an IC_{50} value of 28.31 μ M. This might be due to the presence of a diether substituent present in both the ketonic and aldehydic portion of the compound. These values suggest that **BE4** can effectively scavenge nitric oxide radicals and provide antioxidant defense against nitric oxide-induced oxidative damage.

Following **BE4**, **BE3**, and **BE5** demonstrated the second and third lowest IC_{50} values of 39.70 μ M and 49.13 μ M respectively. This can be attributed to the presence of -Cl substituents in the A ring of the compound **BE3** and the presence of a diether substituent in **BE5**. Although **BE3** and **BE5** possess a slightly higher IC_{50} value than Gallic acid (IC_{50} =19.53 μ M) the reference standard, they can also serve as a nitric oxide radical scavenger and provide antioxidant defense against nitric oxide-induced oxidative damage.

Compounds **BE7**, IC_{50} value of 75.72 μ M, **BE1**, IC_{50} value of 76.48 μ M, **BE6**, IC_{50} value of 145.0 μ M, and **BE2**, IC_{50} value of 216.4 μ M, all showed higher IC_{50} values when compared to Gallic acid the reference standard hence are poor scavengers of nitric oxide radicals. The chalcone can be ranked in terms of their nitric oxide radical scavenging potential as **BE4 > BE3 > BE5 > BE7 > BE1 > BE6 > BE2**.

For the 2, 4-DNPH coupled products used in the nitric oxide scavenging assay, **BE1A** exhibited the highest scavenging activity, as indicated by its lowest IC_{50} value of 19.06 μ M. This might be because **BE1A** has no substituents and has the least steric hindrance. The IC_{50} value suggests that **BE1A** possesses a potent nitric oxide scavenging ability that is even greater than Gallic acid (IC_{50} =28.31) making it an effective agent for reducing nitric oxide-related oxidative stress.

Following **BE1A**, **BE4A** IC_{50} value of 29.05 μ M, **BE2A**, IC_{50} value of 30.60 μ M, **BE5A** IC_{50} value of 33.85 μ M, and **BE6A** IC_{50} value of 38.45 μ M all have slightly higher IC_{50} than Gallic acid the reference standard, they can also serve as an effective agent for reducing nitric oxide-related oxidative stress. Furthermore, **BE3A** IC_{50} value of 79.31 μ M and **BE7A** IC_{50} value of 498.3 μ M, possess relatively high IC_{50} values, therefore they are poor scavengers of nitric acid radicals. This reduced efficacy might be because they both possess chlorine substituents. Hence, the hydrazones can be ranked in terms of their ability to scavenge nitric oxide radicals as **BE1A > BE4A > BE2A > BE5A > BE6A > BE3A > BE7A**.

The comparison between the IC_{50} values of the chalcone derivatives and their coupled products reveals that the 2, 4-DNPH coupled products generally demonstrate better nitric oxide (NO) scavenging activity compared to the original chalcone analogs. This might be due to the bulky nature of the hydrazone derivatives and the electron-donating capacity the nitrogen in the hydrazone functional group possesses. Notable similarities were observed in the nitric oxide radical scavenging capabilities of chalcones and their hydrazone derivatives when compared to prior research findings. This report aligns with previous findings highlighting the strong scavenging potential of hydrazone derivatives^[26, 27].

Analgesic activity

The compounds were evaluated for their capacity to inhibit red blood cell hemolysis, which happens when excessive fluid accumulates inside cells, causing their membranes to rupture. In a hypotonic solution, water enters cells via osmosis, leading to swelling and eventual hemolysis. Cell injury increases susceptibility to further damage, often caused by free radical-induced lipid peroxidation—a chain reaction in which free radicals attack unsaturated fatty acids in cell membranes, resulting in cellular damage.

Analgesic activity assessments were conducted on a specific subset of synthesized products, namely **BE3**, **BE4**, **BE5**, **BE7**, **BE4A**, and **BE7A**.

Among the chalcone derivatives, **BE4** exhibited the most analgesic capacity, displaying an IC₅₀ value of 2.967 µg/ml, surpassing the reference standard dichlorofenac (IC₅₀ value of 154.7 µg/ml). This heightened activity can be due to the presence of diether substituents in both the aldehydic and ketonic portions of the compound. Additionally, **BE3** demonstrated noteworthy analgesic activity, boasting an IC₅₀ value of 129.1 µg/ml, surpassing the reference standard. This might be because of the chlorine substituent in the A ring of the compound. **BE7** also exhibited noteworthy analgesic capacity with an IC₅₀ value of 131.0 µg/ml surpassing diclofenac the reference standard. This might be attributed to chlorine (-Cl) substituents present in both the aldehydic and ketonic portions of the compound.

Moving to the hydrazone derivatives, **BE7A** demonstrated considerable analgesic potential with an IC₅₀ value of 120.0 µg/ml, outperforming the reference standard dichlorofenac (154.7 µg/ml). Conversely, **BE4A** exhibited poor analgesic activity, evident from its significantly higher IC₅₀ value of 355.1 µg/ml.

Conversely, **BE5A** displayed the least analgesic effect, due to its slightly elevated IC₅₀ value of 186.6 µg/ml. Although **BE5A** displays a slightly higher IC₅₀ value when compared to diclofenac the reference standard, it is important to note that it also possesses moderate analgesic activity. In comparison, chalcone **BE4** and its hydrazone derivative **BE4A**, **BE4** (2.976 µg/ml) exhibited superior analgesic capacity compared to **BE4A** (355.1 µg/ml), the observed decline in efficacy can be attributed to steric factors. However, both **BE7** and **BE7A** showed good analgesic capacity, and the hydrazone derivative demonstrated slightly improved analgesic properties, suggesting that the coupling process enhances the analgesic capacity of this compound.

Significant resemblances were noted in the analgesic activities exhibited by both chalcones and their hydrazone derivatives when compared with previous research findings which documented the strong analgesic activities of chalcones^[28].

Antimicrobial Activity

The results of antimicrobial testing conducted through the agar well diffusion method demonstrated that, across all tested concentrations, the test substance showed no activity against the clinical isolates of *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis*. The inhibitory zones around the wells were indistinguishable from those observed in the negative control plates. These findings are consistent with earlier studies that documented negligible anti-microbial activities for tested chalcone analogs^[5, 10, 29].

4. CONCLUSION

Chalcones were synthesized utilizing a modified Claisen–Schmidt condensation reaction in an alkaline medium using sodium hydroxide as a catalyst. Thereafter, the chalcones were converted to hydrazone using 2, 4- dinitrophenyl hydrazine *via* an acid-catalyzed condensation reaction. The chalcone products and their coupled hydrazone products were then characterized and assessed for their antioxidant potential, antimicrobial activity, and analgesic properties. For the antioxidant activity, the chalcone analogs demonstrate superior activity in the DPPH scavenging assay and total antioxidant capacity compared to their 2, 4-DNPH coupled counterparts. Conversely, for the nitric oxide radical scavenging activity, the 2, 4-DNPH-coupled product exhibits stronger scavenging activity in comparison to its parent chalcones. In addition,

the chalcones exhibit enhanced analgesic activity relative to their hydrazone derivatives. Furthermore, the antimicrobial screening indicated no activity against tested bacterial strains.

5. RECOMMENDATION

Molecular docking studies should be conducted to evaluate how these compounds interact with their target receptors, and in *vivo* assays and toxicity profiling should be performed to pave the way for potential clinical trials.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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