

Synthesis, Characterization and Antimicrobial Studies of Four Chalcone Derivatives

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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF FOUR CHALCONE DERIVATIVES.

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ABSTRACT

The increasing prevalence of antimicrobial resistance has intensified the search for structurally simple and biologically active compounds with therapeutic potential. In this study, four chalcone derivatives (A, B, C and D) were synthesized via Claisen–Schmidt condensation using substituted aromatic aldehydes and acetophenone under mild alkaline conditions. The synthesized compounds were obtained in good yields (73–81.9%), with masses ranging from 0.58–0.65 g and melting point ranges of 71–206 °C. The compounds were purified and confirmed by thin-layer chromatography and melting point determination, while structural elucidation was achieved using Fourier Transform Infrared (FT-IR) spectroscopy, with spectra recorded over the range of 650–4000 cm⁻¹, and Gas Chromatography–Mass Spectrometry (GC–MS), which confirmed molecular ions at *m/z* 223.0, 242.2, 222.0 and 251.0 for compounds A, B, C and D, respectively. The compounds were evaluated for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium*, and antifungal activity against *Candida albicans* using the agar well diffusion method. All derivatives exhibited antimicrobial activity, producing inhibition zones ranging from 12–22 mm, with minimum inhibitory concentrations (MICs) of 3.13–50 mg/mL. Compound A (4-amino-substituted chalcone) demonstrated the highest antimicrobial potency, exhibiting the largest zones of inhibition (up to 22 mm against *Escherichia coli*), the lowest MIC values (3.13–12.5 mg/mL), and bactericidal activity at 25–50 mg/mL. The enhanced activity is attributed to the electron-donating amino substituent, which likely improves interactions with microbial targets.

KEYWORD: Chalcones, Synthesis, Antimicrobial studies, TLC, FT-IR, GC-MS.

1. Introduction

The increasing incidence of antibiotic-resistant microbes are a pressing global health risk undermining the efficacy of conventional antibiotics against widespread microbes and leading to extended illness, rising health costs, and higher mortality [1]. This necessitates the discovery of new, effective antimicrobial drugs. Chalcones, for their easy accessibility for synthesis and structural simplicity, represent an attractive target structure for lead structure discovery [2].

Chalcones are important group of organic compounds that are both naturally occurring and synthetically accessible. Structurally, they are characterized by an α,β -unsaturated carbonyl system (enone group) that links two aromatic rings [9]. This basic skeleton is significant because chalcones are known intermediates in the biosynthesis of flavonoids and isoflavonoids in plants [10]. In nature, they play roles in pigmentation, plant growth

regulation, and defense against microbial invasion [5]. They exhibit antimicrobial, antioxidant, anti-inflammatory, anticancer, antimalarial and antiviral activities [6-8]. Chalcones are commonly synthesized via the Claisen–Schmidt condensation reaction, where an aromatic aldehyde reacts with an acetophenone under basic conditions, typically using sodium hydroxide (NaOH) or Potassium Hydroxide (KOH) [3-4]. This reaction produces the (E)-isomer of the Chalcone, which is more stable [11].

2.0 Method

2.1 General Method for the Synthesis of Chalcones (A–D)

The chalcone derivatives (A–D) were synthesized via a base-catalyzed Claisen–Schmidt condensation between equimolar quantities (3.3 mmol) of acetophenone and each of 4-Aminoacetophenone, 4-chlorobenzaldehyde, p-Tolualdehyde (4-methylbenzaldehyde), and 4-dimethylaminobenzaldehyde under mild alkaline conditions, following established procedures [12,13] in a 100 mL conical flask equipped with magnetic stirring. The reaction mixture was maintained at ambient temperature (25–27 °C). Aqueous potassium hydroxide solution (0.72 M) was added dropwise with continuous stirring. The reaction proceeded rapidly with the formation of a precipitate indicating completion of the condensation reaction.

The progress of the reaction was monitored by Thin Layer Chromatography (TLC) on silica gel plates using n-hexane: ethyl acetate (7:3) as the mobile phase. A single spot was observed under UV illumination, confirming the formation of a single product.

The resulting solid was isolated by vacuum filtration using a Büchner funnel, washed with cold ethanol to remove residual impurities, and dried in a desiccator over anhydrous calcium chloride to afford the respective chalcones derivatives A-D in good yield.

2.2 Spectral Analysis

The synthesized chalcone derivatives were characterized using Fourier Transform Infrared (FT-IR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC–MS) to confirm functional group integrity and molecular structure.

FT-IR spectroscopy was employed to identify the characteristic functional groups present in the synthesized compounds. The spectra were recorded within the range of 650–4000 cm^{-1} using the KBr disc method. Each sample was finely triturated with spectroscopic-grade potassium bromide and compressed into a transparent pellet prior to analysis. Spectral acquisition was carried out using a PerkinElmer Spectrum 100 FT-IR spectrometer. GC–MS analysis was performed to determine the molecular masses and fragmentation patterns of the synthesized chalcone derivatives. The analysis was conducted using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass selective detector. Helium was employed as the carrier gas at a constant flow rate of 1 mL/min, and 1 μL of the sample supernatant was injected into the system. Chromatographic separation was achieved using a temperature-programmed oven method in which the initial oven temperature was set at 80 °C and increased to 200 °C at a rate of 15 °C/min. The temperature was then further ramped from 200 °C to 280 °C at 5 °C/min and maintained isothermally at 280 °C for 5 minutes to ensure complete elution of the analytes. Mass spectral detection was carried out under electron ionization (EI) mode at an ionization energy of 70 eV, with the ion source temperature maintained at 230 °C. The obtained mass spectra displayed molecular ion peaks (M^+) corresponding to the expected molecular weights of the chalcone derivatives. Structural assignments were confirmed by comparison of the acquired spectra with entries in the

National Institute of Standards and Technology (NIST) mass spectral database. The combined spectroscopic data conclusively support the successful synthesis and structural validity of the target chalcone derivatives.

2.3 Biological Studies

Introduction to Antimicrobial Evaluation

Antimicrobial resistance is a major global health challenge due to the declining effectiveness of many conventional antibiotics and antifungal agents [14]. The rapid emergence of multidrug-resistant strains has increased the need for structurally novel antimicrobial agents. Chalcones are naturally occurring and synthetic flavonoid precursors that possess diverse biological activities, including antibacterial and antifungal properties [15,16]. Their biological activity is largely attributed to the presence of the reactive α,β -unsaturated carbonyl (enone) system, which plays an important role in interactions with microbial cellular targets [15]. The antimicrobial potential of the synthesized chalcone derivatives was evaluated through in vitro studies conducted at the Department of Pharmaceutical

Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The compounds were tested against selected clinically relevant microorganisms, including: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*, *Candida albicans*. These organisms were selected to evaluate broad-spectrum antimicrobial activity, as Gram-positive and Gram-negative bacteria differ in cell wall architecture, which influences drug permeability and susceptibility [17]. *Candida albicans* was included as a representative opportunistic fungal pathogen commonly associated with systemic and superficial infections [18]. The antimicrobial activities of the synthesized compounds were compared with standard reference drugs: Ciprofloxacin as the antibacterial control and Terbinafine as the antifungal control [19,20]. The evaluation provides preliminary insight into the antimicrobial relevance of the synthesized chalcone framework.

2.4 Determination of Zone of Inhibition (Agar Well Diffusion Method)

The antibacterial activity of the synthesized chalcone derivatives was evaluated using the agar well diffusion technique in accordance with established antimicrobial susceptibility testing guidelines [21]. Sterile nutrient agar was prepared according to the manufacturer's instructions, autoclaved, and aseptically poured into sterile Petri dishes. After solidification, the plates were uniformly inoculated with freshly prepared microbial suspensions standardized to 0.5 McFarland turbidity standard to ensure uniform inoculum density ($\approx 1 \times 10^8$ CFU/mL) [21,22]. Using a sterile cork borer, wells of 6 mm diameter were aseptically punched into the inoculated agar plates. The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain a final concentration of 50 mg/mL. An aliquot of 50 μ L of each test solution was carefully introduced into the respective wells. The plates were allowed to stand at room temperature to permit pre-diffusion of the test compounds into the agar medium. Subsequently, the plates were incubated at 37 °C for 24 hours [21]. After incubation, antimicrobial activity was assessed by measuring the diameter of the clear zones of inhibition surrounding each well using a calibrated ruler. Zone diameters were recorded in millimeters (mm). DMSO served as the negative control, while standard antimicrobial agents were included as positive controls. All assays were performed under aseptic conditions.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the synthesized chalcone derivatives was determined using the broth dilution method in accordance with standard antimicrobial susceptibility testing guidelines [23].

Sterile nutrient broth was prepared and dispensed into sterile test tubes. The synthesized compounds were initially dissolved in dimethyl sulfoxide (DMSO) and further diluted with nutrient broth to obtain a stock concentration of 50 mg/mL. Serial two-fold dilutions were subsequently prepared in nutrient broth to obtain decreasing concentrations of the test compounds.

Fresh microbial suspensions were prepared and adjusted to 0.5 McFarland turbidity standard (approximately 1×10^8 CFU/mL), then further diluted to obtain the appropriate inoculum density for broth dilution assay [23, 24]. A standardized volume of the inoculum was introduced into each tube containing the different concentrations of the test compounds. A growth control (broth + inoculum without test compound) and a sterility control (broth only) were included. The tubes were incubated at 37 °C for 24 hours. Following incubation, the tubes were examined for visible turbidity. The MIC was defined as the lowest concentration of the test compound that showed no visible growth when compared with the growth control [23].

2.6 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC of the synthesized chalcone derivatives were determined from the MIC assay according to standard antimicrobial susceptibility guidelines [25]. Following MIC determination, aliquots (0.1 mL) were aseptically taken from tubes showing no visible growth and subcultured onto freshly prepared sterile nutrient agar plates. The plates were incubated at 37 °C for 24 hours. The MBC was defined as the lowest concentration of the compound that showed no visible colony growth on the agar plate after incubation, indicating bactericidal activity [25]. For antifungal evaluation, aliquots from MIC tubes corresponding to *Candida albicans* were subcultured onto sterile agar medium and incubated under appropriate conditions. The MFC was defined as the lowest concentration at which no fungal growth was observed after incubation [26]. All procedures were carried out under aseptic conditions with appropriate controls.

3.0 RESULTS

3.1 Synthesis of (E)-3-(4-aminophenyl)-1-phenylprop-2-en-1-one (A)

Compound A was obtained as yellow crystalline solid in 78.00% (0.58 g) yield with a melting point of 204–206 °C, indicating good purity and crystallinity consistent with conjugated chalcone systems. The FT-IR spectrum of compound A exhibited a distinct absorption band at 3440 cm^{-1} corresponding to the N–H stretching vibration of the para-amino ($-\text{NH}_2$) substituent on the aromatic ring. The presence of this band confirms successful incorporation of the amino functional group into the chalcone framework. A strong absorption band observed at 1650 cm^{-1} is characteristic of the conjugated carbonyl ($\text{C}=\text{O}$) stretching vibration of the α,β -unsaturated ketone system. The lowering of the carbonyl frequency relative to unconjugated ketones is attributed to resonance stabilization arising from extended π -conjugation across the enone system.

and aromatic rings. The aromatic C–H stretching vibration was observed at 3050 cm^{-1} , confirming the presence of aromatic moieties. In addition, the aromatic C=C skeletal vibrations are expected within the $1580\text{--}1600\text{ cm}^{-1}$ region, consistent with the phenyl rings present in the structure. The combined spectral features support the formation of a conjugated chalcone system bearing a para-amino substituent. The presence of both the N–H stretching band and the conjugated carbonyl absorption conclusively establishes the structural integrity of (E)-3-(4-aminophenyl)-1-phenylprop-2-en-1-one. The GC–MS spectrum of compound A displayed a molecular ion peak at $m/z = 223$, corresponding to the expected molecular weight of $\text{C}_{15}\text{H}_{13}\text{NO}$. The presence of a clear molecular ion peak confirms the molecular composition of the synthesized compound. The spectroscopic data obtained from FT-IR and GC–MS analyses are in full agreement with the proposed structure of compound A. The presence of the amino functionality, conjugated carbonyl system, and aromatic framework confirms successful synthesis via Claisen–Schmidt condensation. The relatively high melting point ($204\text{--}206\text{ }^{\circ}\text{C}$) further supports the formation of a highly conjugated, crystalline α,β -unsaturated ketone. Structure, FTIR and GC–MS spectra are represented in figures 1, 2 and 3.

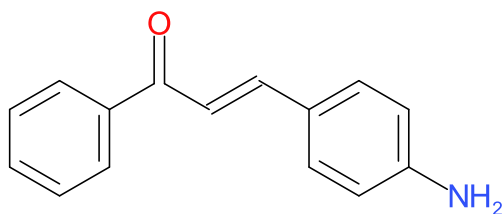
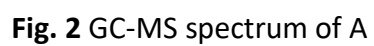
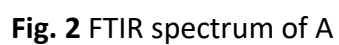


Fig. 1 (E)-3-(4-aminophenyl)-1-phenylprop-2-en-1-one



3.2 Synthesis of (E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (B)

Compound B was obtained as a cream-colored crystalline solid in 81.25% (0.65 g) yield with a melting point of 113–114 °C. The sharp melting range indicates good purity and successful crystallization of the conjugated enone system. FT-IR Spectral Analysis The FT-IR spectrum of compound B revealed a strong absorption band at 1656.8 cm^{-1} , characteristic of the conjugated carbonyl ($\text{C}=\text{O}$) stretching vibration of the α,β -unsaturated ketone system. The observed carbonyl frequency is consistent with chalcone derivatives and reflects resonance stabilization due to π -conjugation across the enone linkage and aromatic rings. A prominent band at 1604.6 cm^{-1} corresponds to the aromatic $\text{C}=\text{C}$ stretching vibration, confirming the presence of the phenyl moieties. The position of this band further supports extended conjugation within the chalcone framework. The absorption band observed at 717.5 cm^{-1} is attributed to the $\text{C}-\text{Cl}$ stretching vibration of the para-chloro substituent. The presence of this characteristic band confirms successful incorporation of the chlorine atom onto the aromatic ring. The electron-withdrawing inductive effect ($-I$ effect) of chlorine slightly influences the electron density distribution across the conjugated system, contributing to subtle shifts in vibrational frequencies.

The combined IR data confirm the presence of a conjugated enone system bearing a para-chloro aromatic substituent, consistent with the proposed structure. The GC–MS spectrum of compound B exhibited a molecular ion peak at $m/z = 242.2$, corresponding to the expected molecular weight of $\text{C}_{15}\text{H}_{11}\text{ClO}$. The presence of this molecular ion peak confirms the molecular composition of the synthesized compound. The spectroscopic data obtained from FT-IR and GC–MS analyses are fully consistent with the proposed structure of (E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one. The presence of the conjugated carbonyl group, aromatic system, and para-chloro substituent confirms successful synthesis via Claisen–Schmidt condensation. The molecular ion peak at m/z 242.2 further validates the structural assignment. Structure, FTIR and GC-MS are in figures 4, 5 and 6.

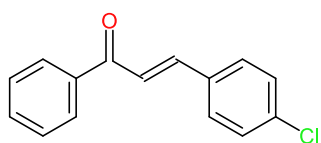


Fig. 4 (E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (B)

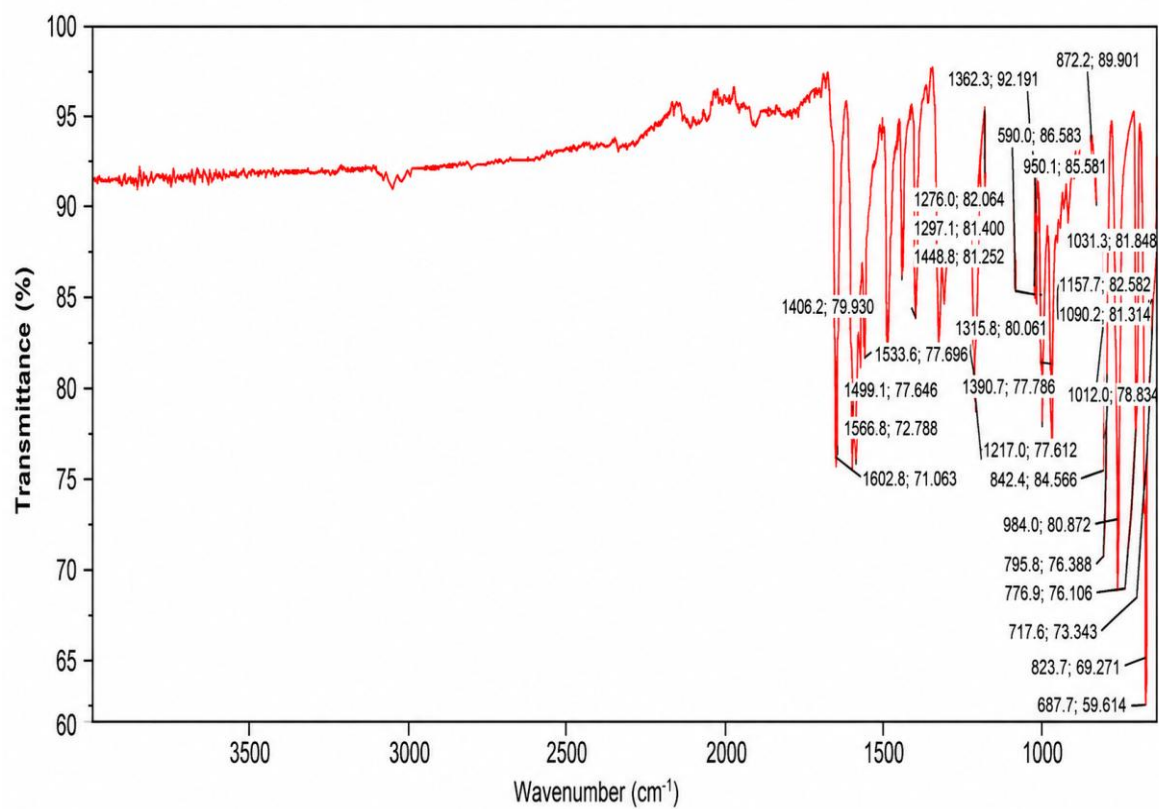


Fig. 6 FTIR Spectrum of

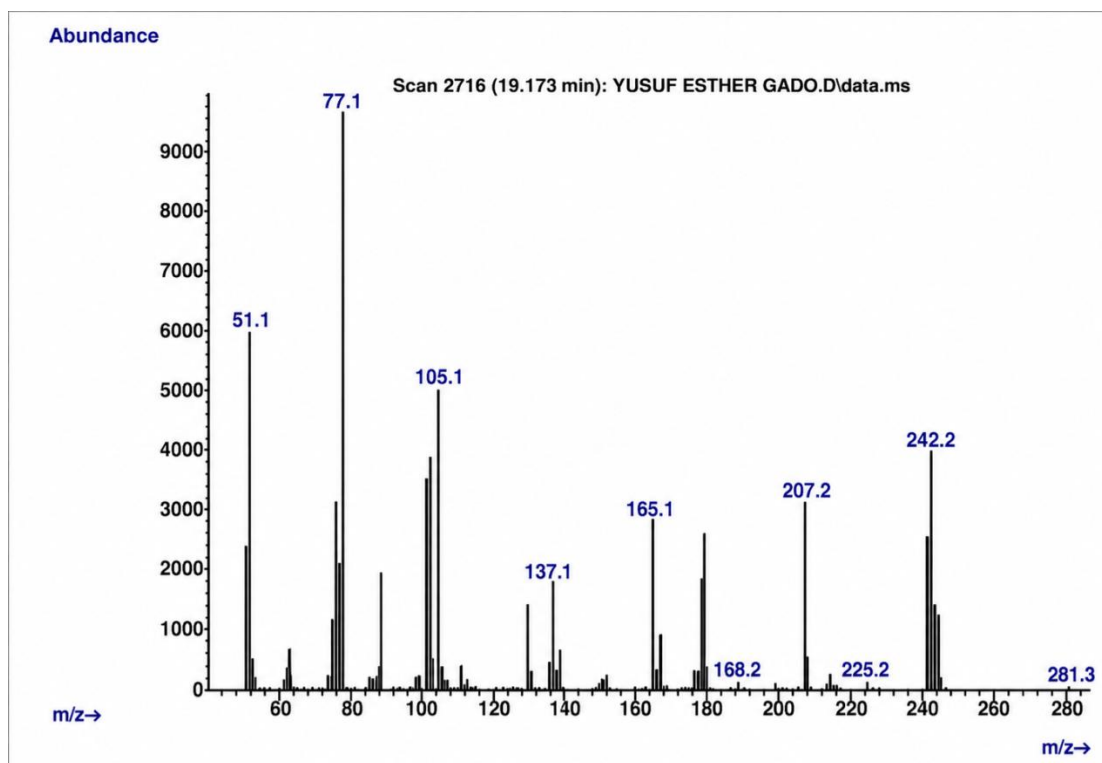


Fig. 6 GC-MS Spectrum of B

3.3 Synthesis of (E)-3-(4-methylphenyl)-1-phenylprop-2-en-1-one (C)

Compound C was obtained as pale yellow crystalline solids in 81.90% yield (0.60 g) with a melting point of 71–75 °C. The relatively lower melting point compared to compounds A and B may be attributed to reduced intermolecular interactions due to the presence of the non-polar methyl substituent. The FT-IR spectrum of compound C exhibited a strong absorption band around 1650 cm^{-1} , corresponding to the stretching vibration of the conjugated carbonyl ($\text{C}=\text{O}$) group of the α,β -unsaturated ketone system. The position of this band is consistent with chalcone derivatives and confirms the presence of an extended π -conjugated enone framework. Aromatic $\text{C}=\text{C}$ stretching vibrations were observed around 1580 cm^{-1} , supporting the presence of the phenyl rings within the structure. The aromatic $\text{C}-\text{H}$ stretching vibration appeared near 3050 cm^{-1} , characteristic of substituted benzene rings.

In addition, a distinct aliphatic $\text{C}-\text{H}$ stretching vibration near 2950 cm^{-1} is attributed to the methyl ($-\text{CH}_3$) substituent located at the para-position of the aromatic ring. The presence of this band confirms successful incorporation of the methyl group. The methyl substituent is an electron-donating group via inductive (+I) effect, which slightly increases electron density over the aromatic system and may influence the electronic distribution across the conjugated enone system. However, the carbonyl stretching frequency remains within the typical chalcone range, indicating preserved conjugation. The IR spectral data collectively confirm the formation of a para-methyl-substituted chalcone derivative. GC-MS analysis of compound C revealed a molecular ion peak at $m/z \approx 222$, corresponding to the expected molecular formula $\text{C}_{16}\text{H}_{14}\text{O}$. The observed molecular ion confirms the molecular weight and structural integrity of the synthesized compound.

Structural Confirmation The combined FT-IR and GC-MS data are in agreement with the proposed structure of (E)-3-(4-methylphenyl)-1-phenylprop-2-en-1-one. The presence of the conjugated carbonyl group, aromatic framework, and para-methyl substituent confirms successful synthesis through Claisen-Schmidt condensation. The molecular ion peak at m/z 222 further validates the assigned molecular structure. Structure, FTIR and GC-MS Spectra are in figures 7, 8 and 9.

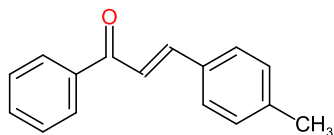


Fig. 7 (E)-3-(4-methylphenyl)-1-phenylprop-2-en-1-one

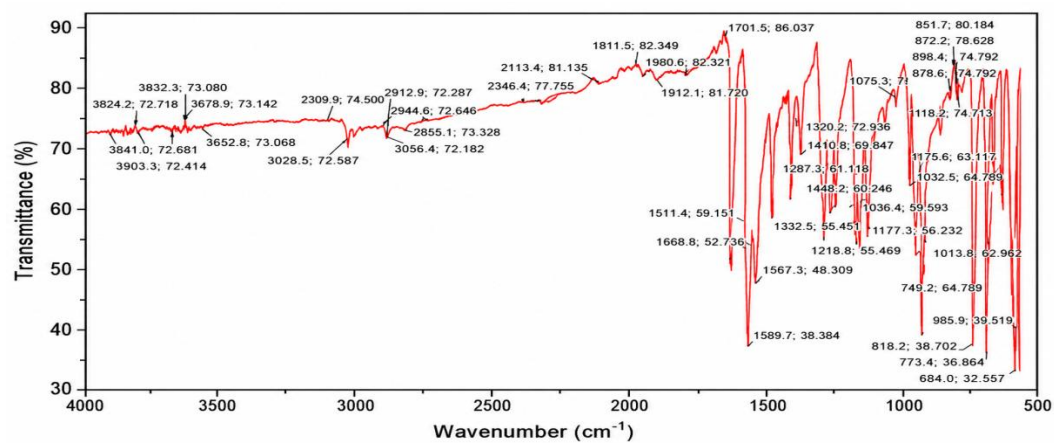


Fig. 8 FTIR spectrum of C

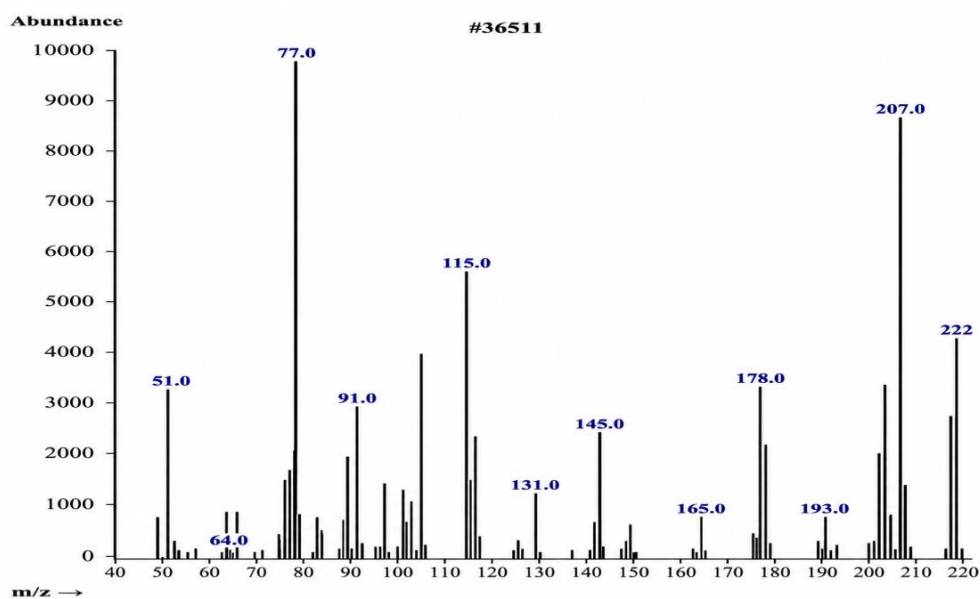


Fig. 9 GC-MS spectrum of c

3.4 Synthesis of (E)-3-(4-dimethylaminophenyl)-1-phenylprop-2-en-1-one (D)

Compound D was obtained as an orange crystalline solid in 73.00% yield (0.61 g) with a melting point of 122–124 °C. The intense orange coloration is consistent with enhanced π -conjugation arising from the strongly electron-donating dimethylamino substituent, which extends the chromophoric system of the chalcone framework. The FT-IR spectrum of compound D displayed a strong absorption band at approximately 1645–1655 cm^{-1} , corresponding to the conjugated carbonyl ($\text{C}=\text{O}$) stretching vibration of the α,β -unsaturated ketone system. The slight lowering of the carbonyl frequency compared to less electron-donating substituents is attributed to increased resonance interaction between the dimethylamino group and the conjugated enone system, resulting in enhanced electron delocalization. Aromatic $\text{C}=\text{C}$ stretching vibrations were observed around 1580–1600 cm^{-1} , confirming the presence of the phenyl rings and extended conjugation across the chalcone backbone.

The aromatic $\text{C}-\text{H}$ stretching bands appeared near 3030–3050 cm^{-1} . In addition, aliphatic $\text{C}-\text{H}$ stretching vibrations corresponding to the methyl groups of the dimethylamino substituent were observed near 2920–2950 cm^{-1} . A characteristic $\text{C}-\text{N}$ stretching vibration of the tertiary amine was observed in the region of 1250–1350 cm^{-1} , supporting the presence of the $-\text{N}(\text{CH}_3)_2$ functional group. The absence of $\text{N}-\text{H}$ stretching bands further confirms that the amino group is tertiary rather than primary or secondary. The observed spectral features are consistent with a para-dimethylamino-substituted chalcone derivative. GC-MS analysis revealed a molecular ion peak at $m/z = 251.0$, corresponding to the expected molecular formula $\text{C}_{17}\text{H}_{17}\text{NO}$. The presence of a stable molecular ion peak reflects strong resonance stabilization across the conjugated α,β -unsaturated carbonyl system enhanced by the electron-donating dimethylamino substituent.

Fragmentation is expected to involve cleavage adjacent to the carbonyl group and possible loss of methyl radicals from the dimethylamino moiety, consistent with typical fragmentation pathways of tertiary amine-containing aromatic compounds.

The combined FT-IR and GC-MS data confirm the successful synthesis of (E)-3-(4-dimethylaminophenyl)-1-phenylprop-2-en-1-one. The presence of the conjugated carbonyl group, aromatic system, and tertiary amine substituent validates the proposed structure. The enhanced conjugation imparted by the strong +M (mesomeric) effect of the dimethylamino group explains both the bathochromic influence and the observed coloration of the compound. Structure, FTIR and GC-MS Spectra are in figures 10, 11 and 12.

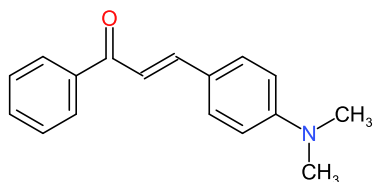


Fig. 10 (E)-3-(4-dimethylaminophenyl)-1-phenylprop-2-en-1-one

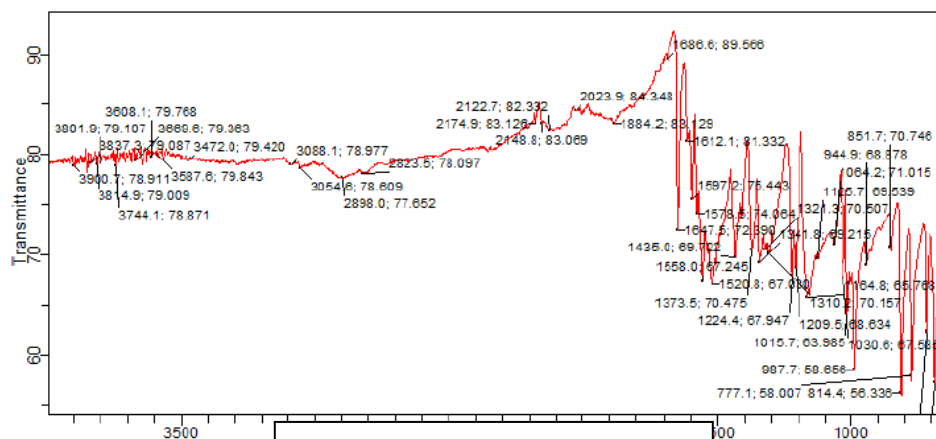


Fig. 11 FTIR spectrum of D

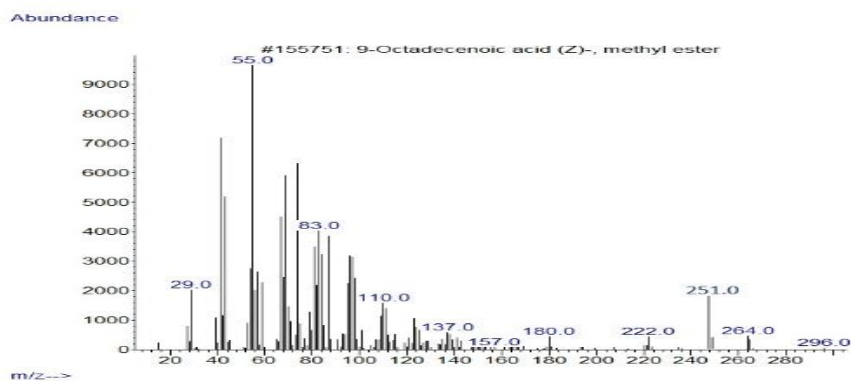


Fig. 12 GC-MS spectrum of D

Table 1: Physical Data of the synthesized chalcones

Sample	M.F	Colour	M. (g)	W.	M.p (°C)	% yield
A	C ₁₅ H ₁₃ NO	Yellow crystalline	223.26		204-206	78.00
B	C ₁₅ H ₁₁ ClO	Cream-coloured crystalline	242.29		113-114	81.25
C	C ₁₆ H ₁₄ O	Pale yellow crystalline	222.27		71-75	81.90
D	C ₁₇ H ₁₇ NO	Orange crystalline	251.32		122-124	73.00

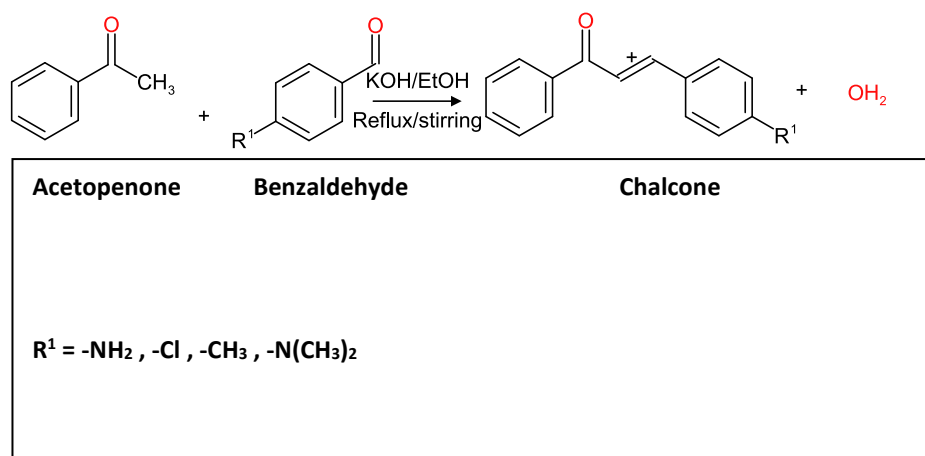


Table 2: Zones of inhibition (mm) at different concentrations

Organisms	Samples and concentrations																Standard Antibiotics	
	50mg/mL				25mg/mL				12.5mg/mL				6.25mg/mL				Ciprofloxacin 10µg/ml	Terbinafine 30µg/ml
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D		
E.C	22	14	1 6	1 3	19	1 2	1 3	0	1 6	0	1 2	0	13	0	0	0	35	-
S.A	20	17	1 5	1 2	18	1 5	1 2	0	1 5	13	0	0	13	12	0	0	37	-
S.T	19	15	1 4	1 2	17	1 3	1 2	0	1 5	0	0	0	12	0	0	0	34	-
B.S	20	16	1 7	1 8	18	1 4	1 5	1 5	1 6	13	1 3	1 4	14	12	1 2	1 2	26	-
C.A	16	12	1 2	1 3	13	0	0	0	0	0	0	0	0	0	0	0	-	20

Table 3 Minimum Inhibitory Concentrations (MIC) (mg/mL)

Organisms	Samples			
	A	B	C	D
E.C	3.1	25.0	25.0	50.0
S.A	6.3	12.5	25.0	50.0
S.T	6.3	25.0	25.0	50.0
B.S	6.3	12.5	12.5	12.5
C.A	12.5	50.0	50.0	50.0

Table 4 Minimum Bactericidal/fungicidal concentrations (MBC/MFC) (mg/mL)

Organisms	Samples			
	A	B	C	D
E.C	25	-	50	-
S.A	25	50	50	-
S.T	25	50	-	-
B.S	25	50	50	50
C.A	50	-	-	-

4.0 Discussion

4.1 Synthesis and Physicochemical Properties

The chalcone derivatives (A–D) were successfully synthesized via base-catalyzed Claisen–Schmidt condensation, yielding crystalline products in good yields ranging from 73–81.9%, indicating an efficient and reproducible synthetic protocol. Chalcones are known to form stable crystalline solids due to their extended π -conjugated systems [27]. The melting points of the compounds varied significantly (71–206 °C), following the order $A > D > B > C$. Compound A exhibited the highest melting point (204–206 °C), which can be attributed to strong intermolecular hydrogen bonding arising from the para-amino ($-\text{NH}_2$) group. In contrast, compound C showed the lowest melting point (71–75 °C), reflecting weaker intermolecular interactions due to the non-polar methyl substituent. Compounds B and D displayed intermediate melting points (113–114 °C and 122–124 °C, respectively), influenced by the electron-withdrawing chlorine substituent and the bulky dimethylamino group. These observations highlight the role of substituent effects on crystal packing and lattice stability [28, 29].

4.2 Spectral Characterization

4.2.1 FT-IR Analysis

The FT-IR spectra of all synthesized compounds exhibited characteristic features consistent with chalcone structures. A strong absorption band corresponding to the α,β -unsaturated carbonyl ($\text{C}=\text{O}$) group was observed within the range of 1645–1656 cm^{-1} , confirming the formation of the conjugated enone system. Additionally, aromatic $\text{C}=\text{C}$ stretching vibrations appeared in the region of 1580–1605 cm^{-1} , while aromatic $\text{C}-\text{H}$ stretching bands were observed around 3030–3050 cm^{-1} , indicating the presence of substituted benzene rings [27].

Substituent-specific bands further supported structural assignments. The $\text{N}-\text{H}$ stretching band ($\sim 3440 \text{ cm}^{-1}$) observed in compound A confirmed the presence of a primary amine group. Compound B exhibited a characteristic $\text{C}-\text{Cl}$ stretching band ($\sim 717 \text{ cm}^{-1}$), while compounds C and D showed aliphatic $\text{C}-\text{H}$ stretching bands (2920–2950 cm^{-1}) corresponding to methyl groups. In compound D, the presence of a $\text{C}-\text{N}$ stretching band (1250–1350 cm^{-1}) and absence of $\text{N}-\text{H}$ vibration confirmed the tertiary amine functionality. These spectral features collectively verify the successful incorporation of the intended substituents into the chalcone framework [28].

4.2.2 GC–MS Analysis

The molecular ion peaks observed in the GC–MS spectra were consistent with the expected molecular weights of the synthesized compounds, confirming their identities:

A: $m/z = 223$ ($\text{C}_{15}\text{H}_{13}\text{NO}$)

B: $m/z = 242.2$ ($\text{C}_{15}\text{H}_{11}\text{ClO}$)

C: $m/z \approx 222$ ($\text{C}_{16}\text{H}_{14}\text{O}$)

D: $m/z = 251.0$ ($\text{C}_{17}\text{H}_{17}\text{NO}$)

These results validate the successful synthesis and purity of the compounds [29].

4.3 Antibacterial Activity

The antibacterial activity of the synthesized chalcones was evaluated against selected Gram-positive and Gram-negative bacteria. All compounds exhibited measurable activity, although with varying degrees of effectiveness. Chalcones are known to possess antimicrobial properties due to their reactive α,β -unsaturated carbonyl system [32].

Compound A showed the highest antibacterial activity, with zones of inhibition ranging from 12–22 mm at different concentrations, indicating strong potency. This enhanced activity can be attributed to the presence of the -NH_2 group, which facilitates hydrogen bonding interactions with microbial targets and improves cellular penetration [33].

Compound C and B exhibited moderate activity (12–17 mm), likely due to increased lipophilicity from the methyl substituent, which enhances membrane permeability and the influence of the electron-withdrawing chlorine substituent respectively. In contrast, compound D demonstrated relatively lower activity (12–18 mm), which may be due to steric hindrance associated with the bulky dimethylamino group despite its strong electron-donating character. However, all compounds were less active than the standard antibiotic (ciprofloxacin), indicating moderate antibacterial efficacy [34].

4.4 Antifungal Activity

The antifungal activity of the compounds against *Candida albicans* revealed generally weak inhibition, with zones ranging from 12–16 mm, compared to the standard drug terbinafine. This observation suggests that the synthesized chalcones exhibit greater selectivity toward bacterial strains than fungal organisms, consistent with previous reports [35].

4.5 Minimum Inhibitory Concentration (MIC)

The MIC values further confirmed the antibacterial potency of the compounds. Compound A exhibited the lowest MIC values (3.13–12.5 mg/mL), indicating the highest activity. Compounds B and C showed moderate MIC values (12.5–50 mg/mL), while compound D displayed the highest MIC values (~50 mg/mL), indicating reduced activity.

These results demonstrate that electron-donating substituents capable of hydrogen bonding significantly enhance antimicrobial efficacy [33].

4.6 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MBC results indicated that compound A possesses strong bactericidal activity at relatively low concentrations (25–50 mg/mL). Compounds B and C required higher concentrations to achieve bactericidal effects, while compound D showed limited bactericidal activity. This suggests that compound A exhibits both bacteriostatic and bactericidal properties, whereas the other compounds are primarily bacteriostatic in nature [32].

5.0 Conclusion

The synthesized chalcone derivatives (A–D) were obtained in good yields ranging from 0.58–0.65 g (73–81.9%), confirming the efficiency of the Claisen–Schmidt condensation. The compounds exhibited melting points within the range of 71–206 °C, reflecting the influence of substituent variation on intermolecular interactions and crystal stability.

5.1 Spectral characterization confirmed the successful formation of the chalcone framework across all derivatives. The FT-IR spectra consistently showed characteristic absorption bands within defined ranges, including 1645–1656 cm^{-1} for the conjugated C=O group, 1580–1605 cm^{-1} for aromatic C=C stretching, 3030–3050 cm^{-1} for aromatic C–H vibrations, and 2920–2950 cm^{-1} for aliphatic C–H stretching. Additional substituent-related absorptions were observed within 1250–1350 cm^{-1} (C–N stretching), ~700–720 cm^{-1} (C–Cl stretching), and ~3400–3450 cm^{-1} (N–H stretching), confirming the successful incorporation of the respective functional groups. These results, supported by GC–MS data, validate the structural integrity of the synthesized compounds [\[27–29\]](#).

5.2 Biological evaluation revealed that all compounds possess antimicrobial activity, with inhibition zones ranging from 12–22 mm. Compound A exhibited the highest antibacterial activity, with MIC values of 3.13–12.5 mg/mL and bactericidal activity within 25–50 mg/mL. Compounds B and C showed moderate activity, while compound D displayed comparatively lower potency. Antifungal activity across the series was generally weak, with inhibition zones ranging from 12–16 mm, indicating a preference toward antibacterial activity.

Abbreviations

TLC: Thin Layer Chromatography
FT-IR: Fourier Transform Infrared Spectroscopy
GC-MS: Gas Chromatography Mass Spectrometry
ZOI: Zone Of Inhibition
MIC: Minimum Inhibitory Concentration
MBC: Minimum bactericidal Concentration
MFC: Minimum Fungicidal Concentration
M.F: Molecular Formular
M.W: Molecular weight
M.p: Melting point
E.C: *Escherichia coli*
S.A: *Staphylococcus aureus*
S.T: *Salmonella typhimurium*
B.S: *Bacillus subtilis*
C.A: *Candida albicans*
mm: millimeter
mg/mL: milligram per milliliter

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Author Contributions

Nasiru yusha'u handled the general synthesis procedure, experimental methods and spectral analysis; Yusuf Esther Gado prepared the abstract, introduction, keywords, abbreviations and references; Aminu Muhammad handled the biological studies including ZOI, MIC and MBC/MFC; Abdulmumeen abdulqoyum durojaye prepared results, discussion and conclusion. All four authors participated in laboratory work and reviewed the manuscript and approved the version. James dama habila supervised the research, provided scientific guidance, reviewed the manuscript and approved the final version

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Data Availability

The datasets generated during and/or analyzed during the current study are available in the [ZENODO] repository, <https://doi.org/10.5281/zenodo.21925529>

DECLARATIONS

Ethics approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare no competing interest

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