

Preparation and Antifungal Properties of Chalcone and Halogenated Derivatives

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Abstract

Acetophenone (1-phenylethan-1-one) reacted with benzaldehyde in the presence of acetic acid, potassium hydroxide, and ethanol on an ice bath for 24 hours via the Claisen-Schmidt condensation reaction giving rise to chalcone (2E-1, 3-diphenylprop-2-en-1-one). On condensation of acetophenone with chlorinated benzaldehyde gives the halogenated derivatives of chalcone. The products were reduced with hydrogen peroxide and nickel reagent to form the epoxide derivatives of the halogenated chalcones (4-chlorochalcone and 4-chlorochalcone epoxide). Structure elucidation of the synthesized compounds was done using elemental analysis, ¹H NMR, ¹³CNMR, FTIR, TLC, and UV/VIS. Synthesized compounds were obtained in high purity and percentage yield. The microbial activity of the synthesized compounds was studied against some test organisms, *Candida albican*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Microbial results showed the synthesized compounds were void of antibacterial activity but showed marked activities against *Candida albican*. Hence are promising molecules in the combat of fungal infections globally.

Keywords: Halogenated Chalcones, Antifungal, Synthesis, Thin-Layer Chromatography.

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INTRODUCTION

Microorganisms on daily basis develop resistance to available conventional antimicrobial agents in clinical practice, thus, the need for the search of more active, effective, less toxic and cost-effective alternatives to already existing medications. Much study has been carried out with the sole aim to discover the therapeutic value of chalcones and different derivatives [1-3]. Chalcones constitute one of the important classes of anticancer agents that have shown promising therapeutic efficacy in the management of human cancers and microbial infections. They are considered as the precursors of flavonoids and isoflavonoids and are abundantly available in edible plants. Substituted chalcones have shown a wide variety of activities, such as anticancer, anti-inflammatory, antifungal, antibacterial, antiparasitic, antiulcer and analgesic activities [4-8]. Several modifications on chalcone chromophore have been reported, these include the hydroxyl, methoxy, and amino groups as substituents with promising anticancer activity and

antimicrobial activities [9-11]. Notably, the 4, 6-diphenyl-2-thiopyrimidine and epoxide derivatives of chalcones had been shown to have remarkable anti-inflammatory properties [12, 13]. These reported properties and the essential role of chalcones derivatives, especially the heterocyclic pyrazoline, pyrimidines halogenated derivatives in the certain biological reaction inspired us to synthesis all these halogenated derivatives of chalcone and further subjected the products for antifungal screening and structural characterization using nuclear magnetic resonance, infra-red spectroscopy, Ultraviolet/Visible spectrophotometry and thin-layer chromatography [14, 15]. Efforts in this study were to synthesize halogenated derivatives of chalcones using the Claisen-Schmidt condensation reaction between different derivatives of halogenated benzaldehyde and acetophenone base with further structural elucidations of the synthesized products that can be used for the formulation of antifungal therapy.

Synthetic Pathway

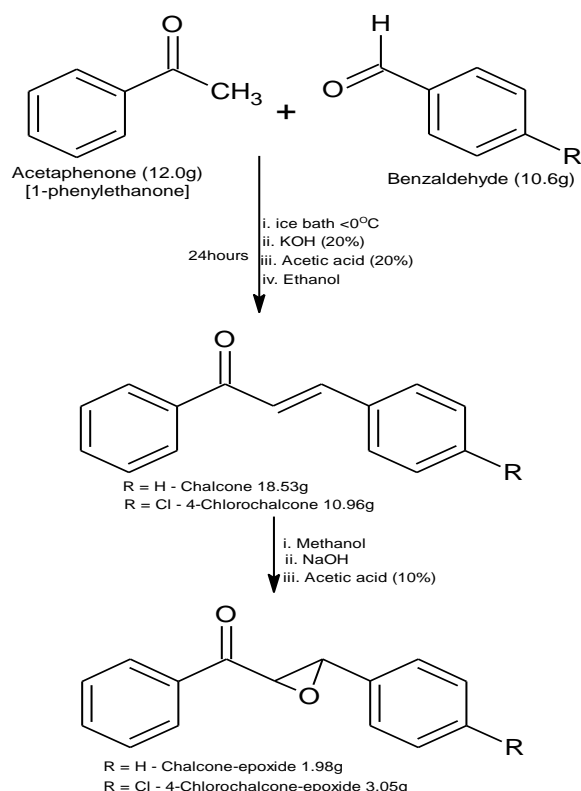


Fig-1: Synthetic pathway of halogenated chalcones (ChemSketch software)

MATERIALS AND METHODS

MATERIALS

Acetophenone, benzaldehyde, *p*-chlorobenzaldehyde, ethyl acetate, petroleum spirit, dimethyl sulfoxide, ethanol, potassium hydroxide, acetic acid, and methanol were procured from BDH chemicals, England. The microbial cultures were obtained from the Faculty of Pharmacy, Niger Delta University. The melting point was quantified with the Gallen-Kamp melting point apparatus while Nuclear Magnetic Resonance and IR data were performed on a 400 MHz instrument, (Varian Inc. Palo Alto, California, USA) and FTIR-8400S equipment (Shimadzu global links, North America).

Preparation

In the synthesis of chalcone, 10.6g and 12.0g of Benzaldehyde and acetophenone respectively were condensed (Claisen-Schmidt condensation) in a round bottom flask inclosing ethanol, 25mL, stirred on an ice bath below 0°C. 20mmL of Potassium hydroxide (20%) was added to the mixture in a drop-wise manner using a burette while the stirring continuous for 15-20 minutes, the mixture was allowed to stand for 24 hours and ice chips were added and were acidified with 25mL of cold acetic acid (20%), by titration, resulting in the formation of some precipitate that was filtered under suction and recrystallized in ethanol medium to get the pure sample (chalcone). It was then air dried, weighed and the melting point was determined. This same procedure was used in the preparation of 4-chlorochalcone (halogenated chalcone) using 4-chlorobenzaldehyde (7.02g) and acetophenone (6.0g). The above products were oxidized in the presence of methanol (60mL), 10% of sodium hydroxide (10mL), 10% acetic acid (20mL) respectively. This resulted in chalcone epoxide and 4-chlorochalcone epoxide. The products were purified using the above methods.

Elemental analysis (Chromatographic analysis, FTIR, NMR, and UV/VIS)

Ethyl acetate and petroleum spirit were used as the solvent system in a 3:7 ratio. TLC chromatograms were activated in an oven for 5 minutes. The tank was allowed to saturate and the synthesized products were spotted against the starting material after dissolving in methanol. The solvent system was allowed to move upwards until it gets to the solvent. The plate was carefully removed and viewed either under UV lamb or an iodine tank (were spots were not visible under UV lamb) and R_f values were determined. The compounds were further subjected to IR, ¹HNMR, ¹³CNMR and UV spectroscopic analysis in a bid to elucidate their structural components and functional groups.

Test Organisms

The test organisms cultures isolates (*Candida albican*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*), were obtained from the microbial bank of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Nigeria. The bacterial isolates were standardized by colony suspension and matching the strain's suspension with 0.5 McFarland standard to give a final concentration of 1.5×10^8 cfu/mL.

Determination of Zone of Inhibition (ZI)

The zones of inhibition were measured in millimeters and compared with the control standards using the method described by Bonev *et al.*, [16]. The antibiotic susceptibility testing was determined using Kirby-Bauer modified diffusion technique, which involves swabbing Mueller–Hinton agar (MHA), plates with the resultant saline suspension of each strain and six wells were aseptically bored using size 6mm cork borer on the agar surfaces seeded with the test organisms. The wells were sealed at the bottom with molten sterilized agar. Stock solutions of the synthesized compounds were prepared by dissolving 500mg of each in 5mL DMSO to give a concentration of 100000mcg/mL. Then 0.1, 0.05, 0.025 and 0.0125 mL of the stock corresponding to 10000, 5000, 2500 and 1250 mcg/mL respectively, of the synthesized compounds were aseptically introduced into the agar wells, and same concentrations of ketoconazole antifungal standard were prepared aseptically and transferred to the wells. The gentamicin and ciprofloxacin antibacterial discs were also placed on the agar surface. The plates were left undisturbed on the bench for 30 minutes to enable the agar to absorb the compounds adequately, incubated for 24 hours at 37°C and observed for confluent growth of the microorganisms and clear zones of inhibition around the samples in the agar wells.

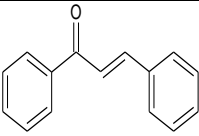
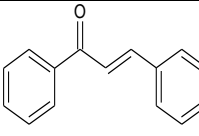
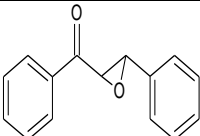
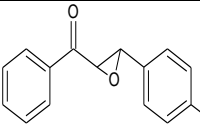
Data Analysis

Data were analyzed with Graphpad Instat version 3.0 statistical software. All analyzed data were expressed as mean \pm standard deviations (SDs). One-way ANOVA and Tukey-Kramer multiple comparisons test were performed to analyze the differences between test and control groups ($P < 0.05$).

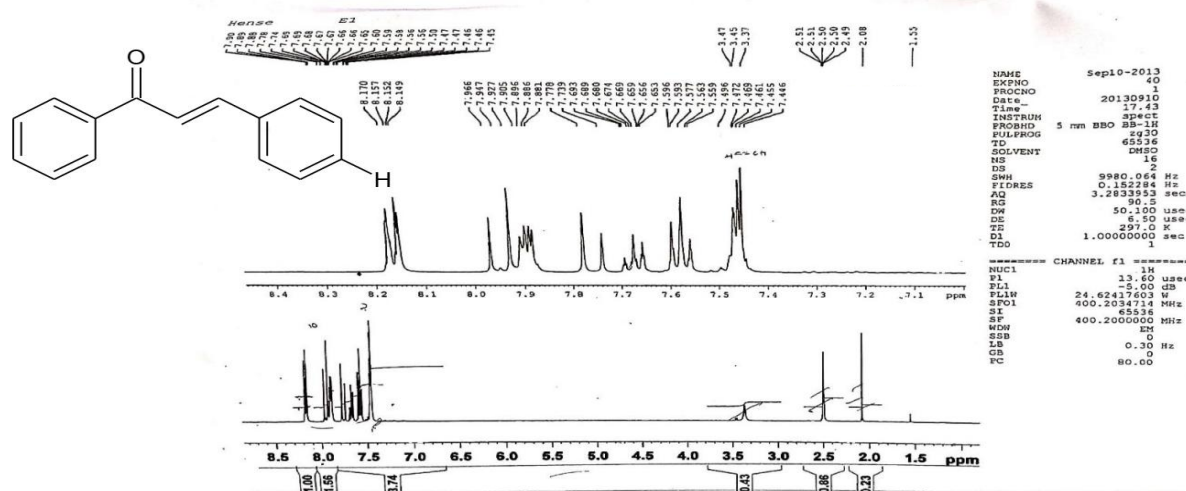
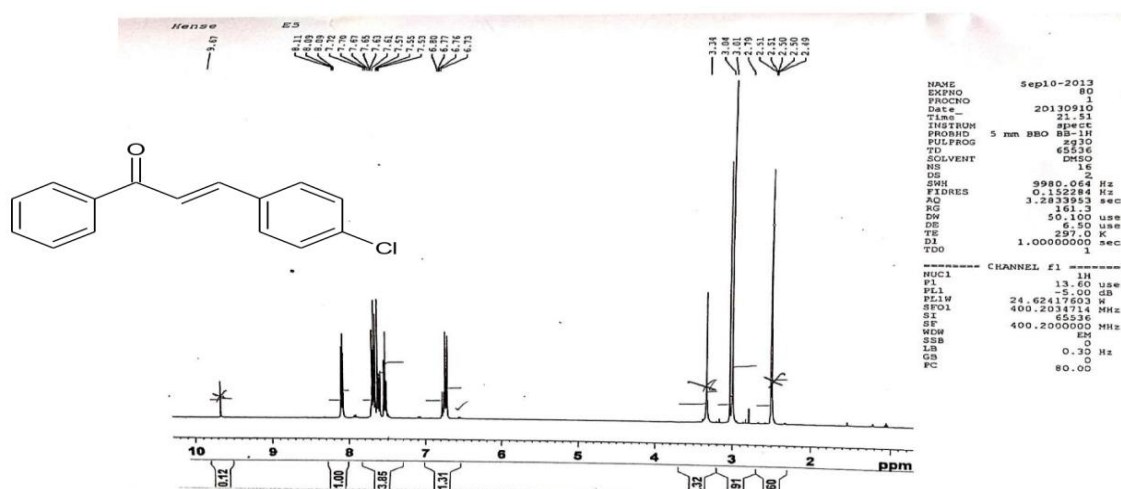
RESULTS

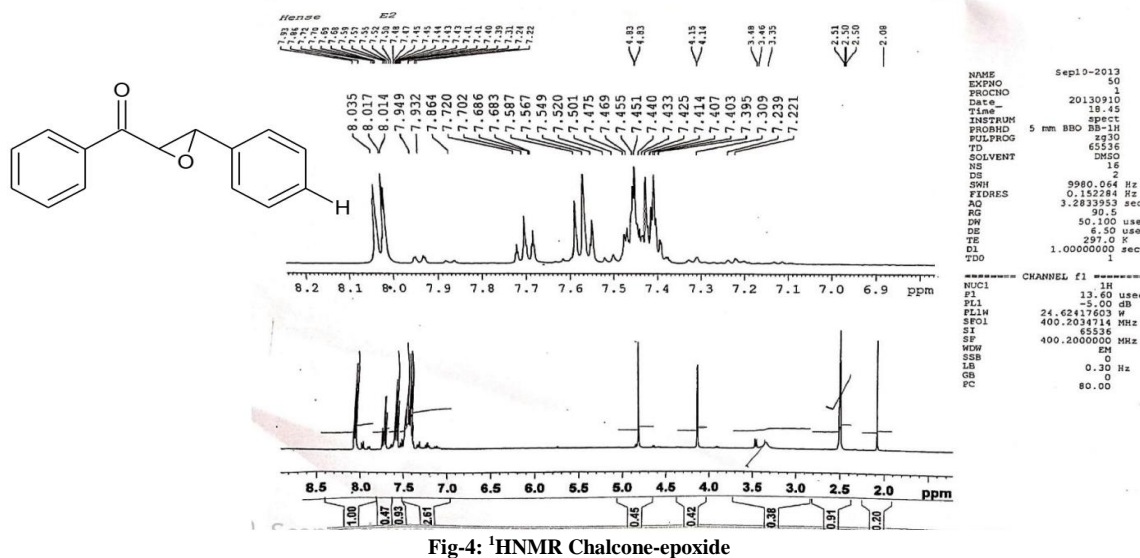
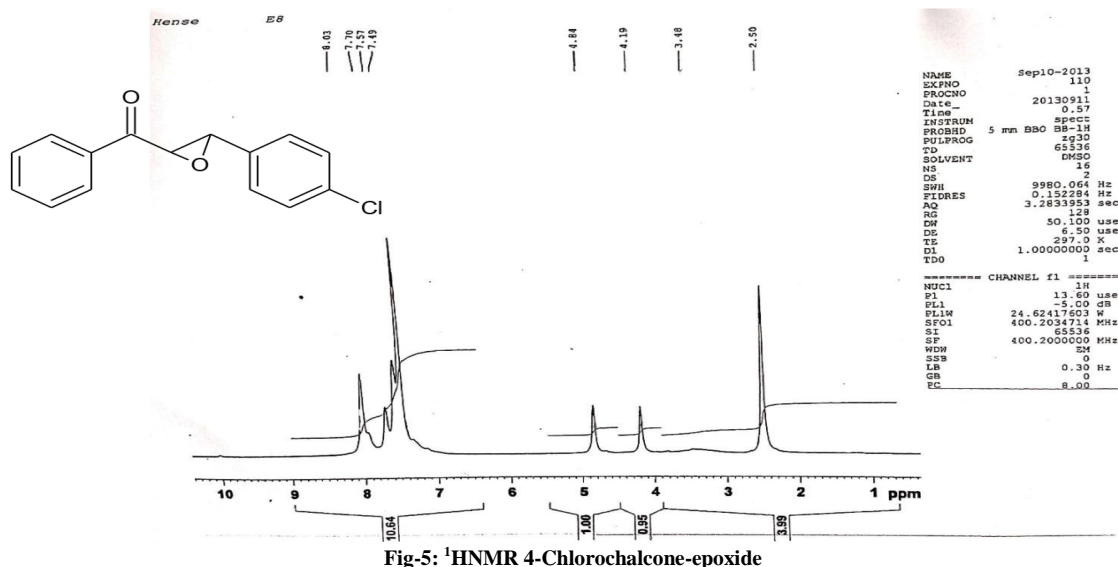
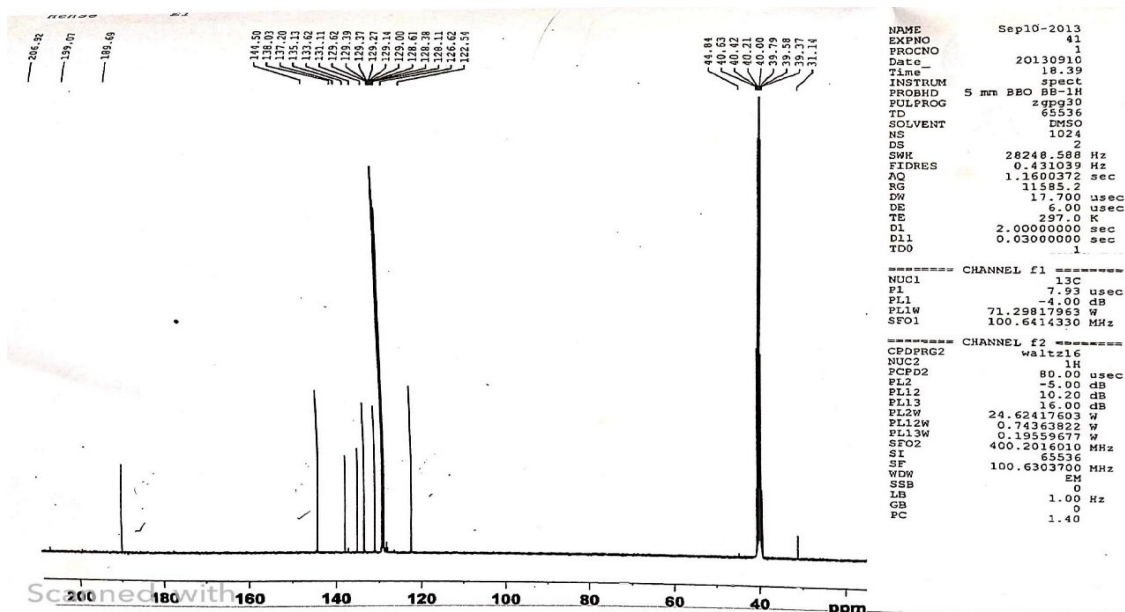
The prepared compounds were analyzed chemically with TLC, FTIR, and NMR while biological assay was carried out with microorganisms as shown in the tables below.

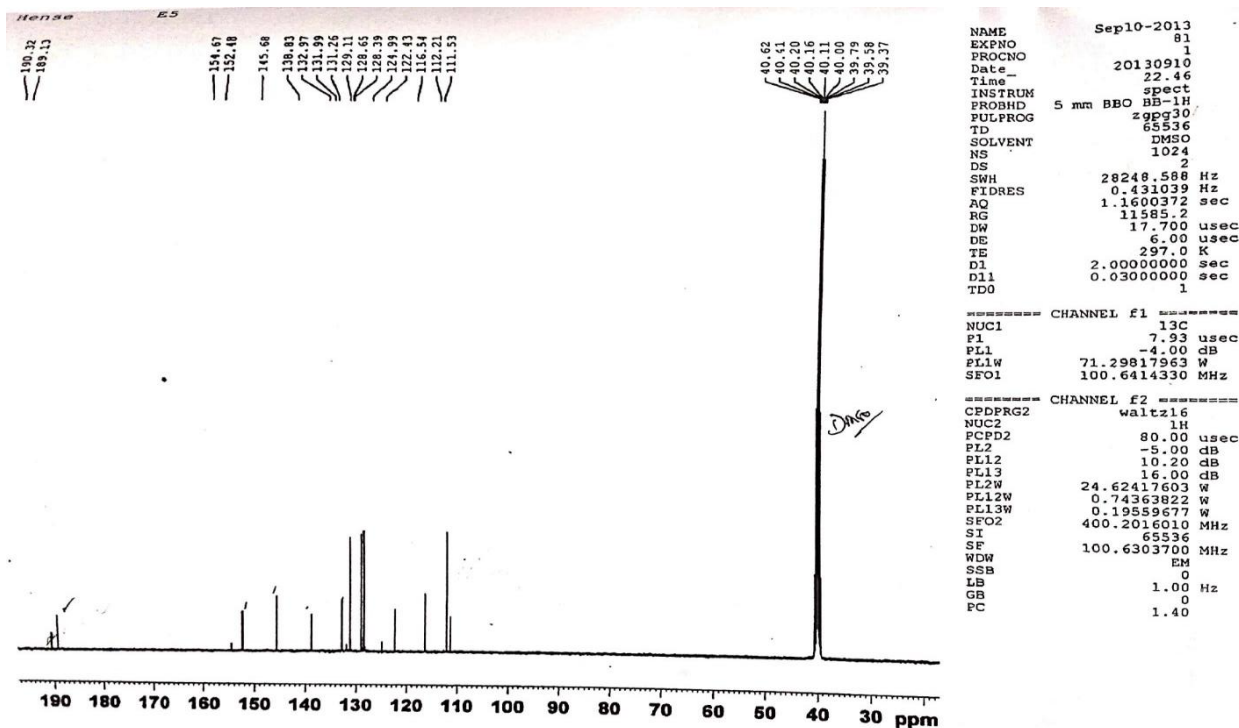
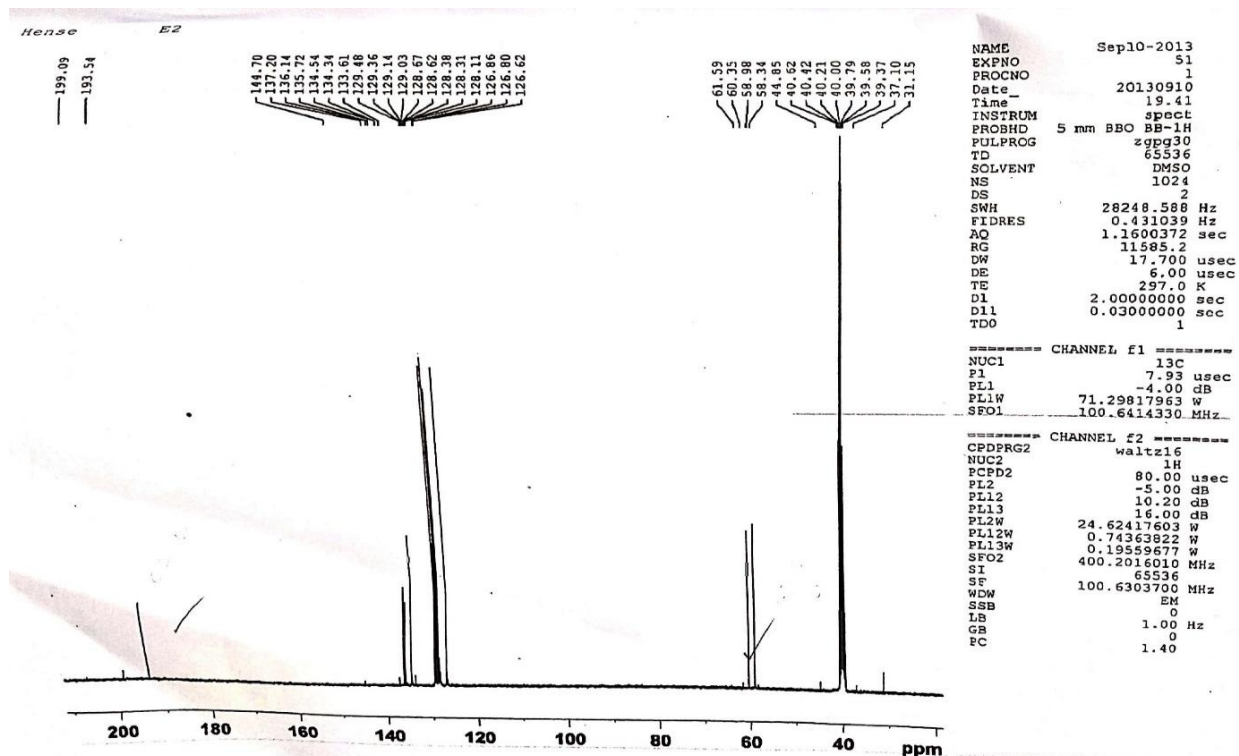
Table-1: Prepared Chalcone and derivatives physicochemical and elemental analysis

Sample	Chalcone	4-chloro-chalcone	Chalcone-epoxide	4-chloro-chalcone-epoxide
Structure				
IUPAC name	(2E)-1,3-diphenylprop-2-en-1-one	(2E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one	Phenyl(3-phenyloxiran-2-yl)methanone]	[3-(4-chlorophenyl)oxiran-2-yl](phenyl)methanone
Yield (%)	18.53g (89.10)	10.96g (90.38)	1.98g (44.20)	3.05g (58.90)
Melting point	43-47°C	90-99°C	64-68°C	54-58°C
Rf value	0.69	0.56	0.72	0.64
TLC spots	1	1	1	1
FTIR (KBr) - Peak, intensity (cm⁻¹)				
C = O	1654.9 (22.62)	1645.33 (35.6)	1681.98 (34.1)	1656.91 (36.57)
C = C (Ar)	1589.4 (10.22)	1543.1 (21.7)	1575.89 (48.96)	1579.75 (40.62)
C=C (Alkene/alkane)	3055.35 (16.4)	3084.28 (22.7)	3045.70 (44.32)	3059.2 (38.23)
C – Cl	-	792.77 (45.34)	-	698.25 (43.71)
C – O	-	-	1236.41 (46.37)	1093.67 (44.82)
¹ HNMR (DMSO d ₆) δppm	7.45 (t, j = 2H, Ar-H, gg); 7.57 (t, j = 2H, Ar-H, ff); 7.65-7.69 (m, 2H, Ar-H, ee),	7.52-8.17 (d, Ar-H); 6.7-6.9 (d, HC-C).	4.15 (d, j = 4Hz, H-CO); 4.83 (d, j = 4Hz, H-CO);	3.34 (s, HC-O); 3.82 (s, HC-O); 7.4-8.03 (Ar-H)

	7.74-7.78 (d, $j = 15.6$, 1H, =CH, d); 7.88-7.90 (m, 2H, Ar-H, aa); 7.93-7.97 (d, $j = 15.6$, 1H, =CH, e); 8.15-8.17 (m, 2H, Ar-H, bb)		7.39 – 8.03 (Ar-H)	
$^{13}\text{CNMR}$ (DMSO d_6) δppm				
Ar - C = C	129 – 138	110 – 130	126 – 137	128 – 135
Alkene - C = C	144	140	-	-
C = O	190	190	193	198
C – O	-	-	56 – 60	58 – 60
UV-VISIBLE (Methanol) λ_{max}				452.00 – 0.153
				432.00 – 0.070
				342.00 – 0.262
				252.00 – 0.213

 ^1H NMR and ^{13}C NMR Spectrum of Synthesized CompoundsFig-2: ^1H NMR ChalconeFig-3: ^1H NMR 4-Chlorochalcone


Fig-4: ¹H NMR Chalcone-epoxide

Fig-5: ¹H NMR 4-Chlorochalcone-epoxide

Fig-6: ¹³C NMR Chalcone


Fig-7: ¹³CNMR 4-Chloroachalcone

Fig-8: ¹³CNMR Chalcone-epoxide

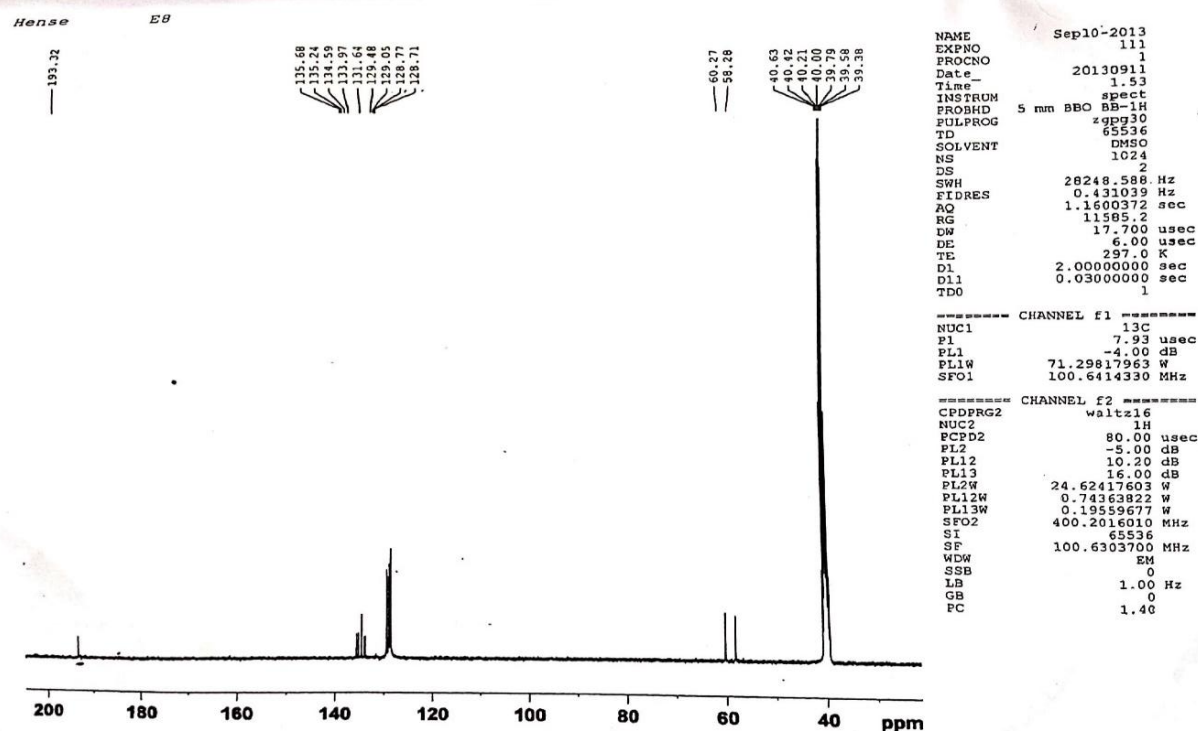
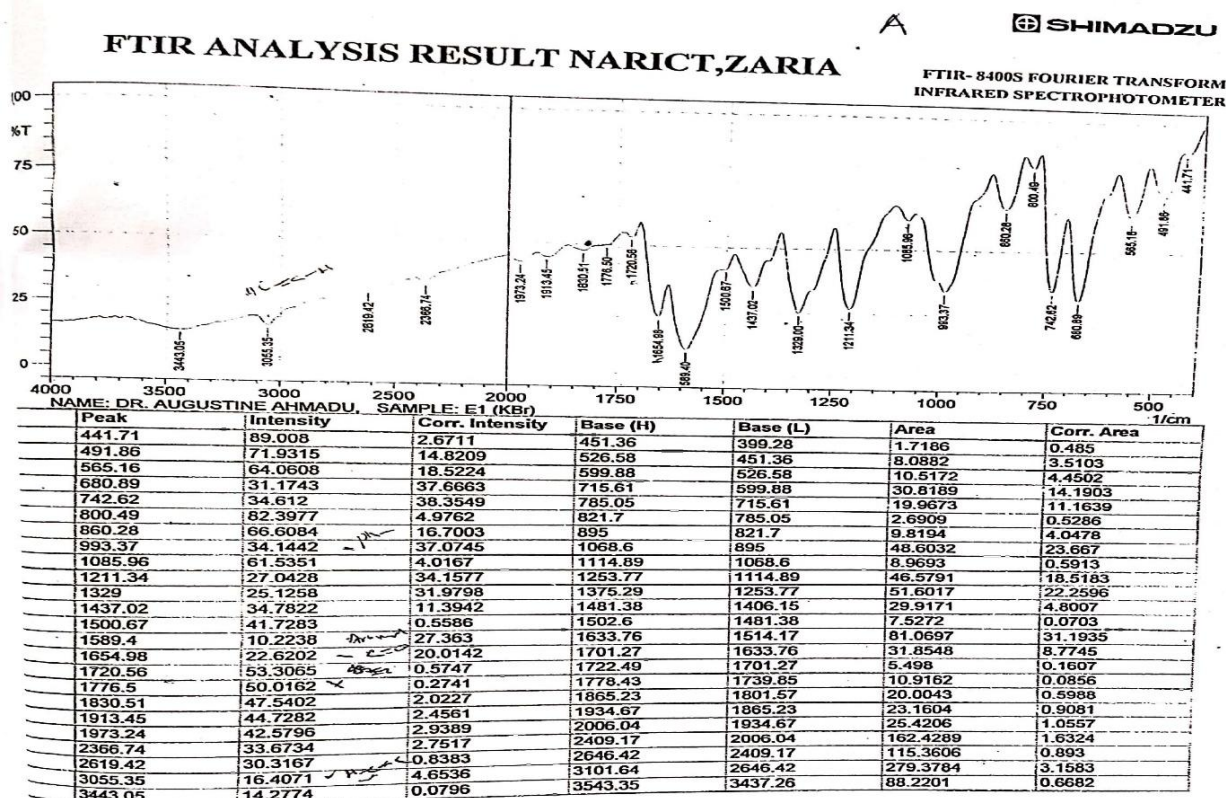

Fig-9: ¹³CNMR 4-Chlorochalcone-epoxide


Fig-10: FTIR – Chalcone

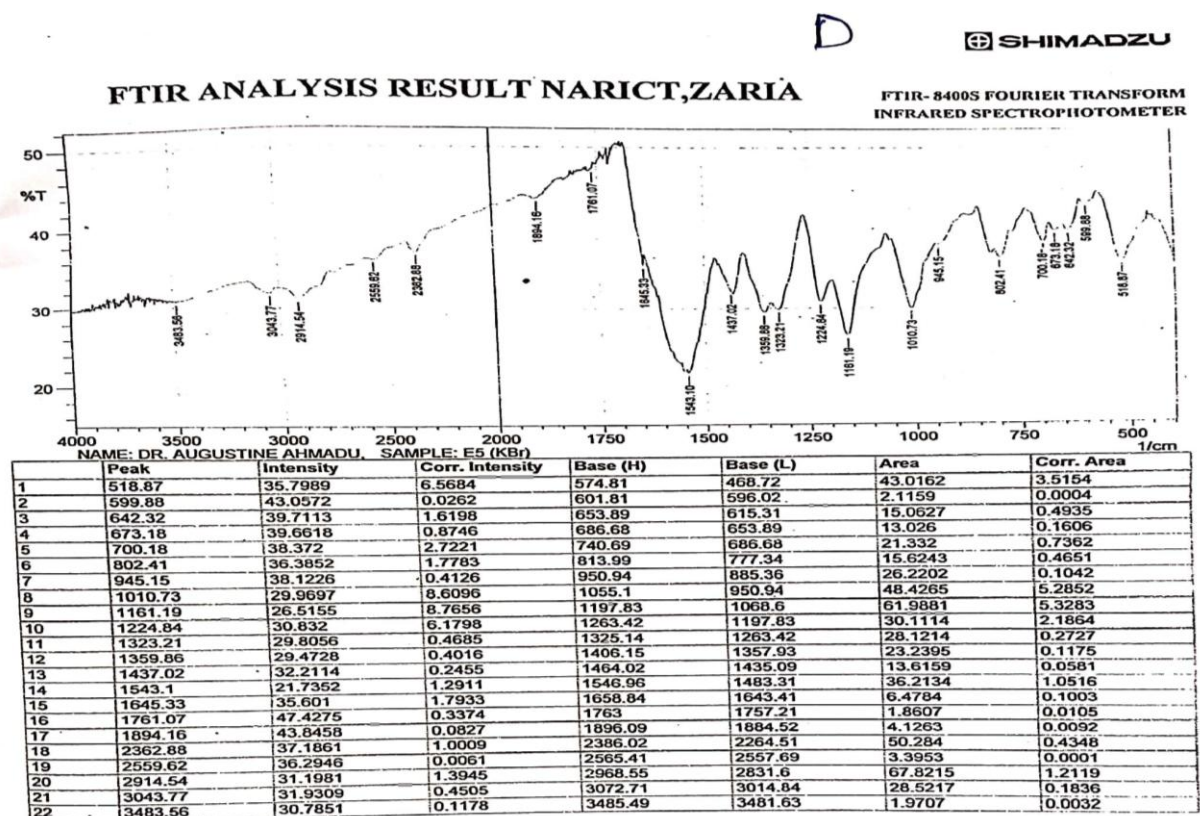


Fig-11: FTIR – 4-Chlorocholeone

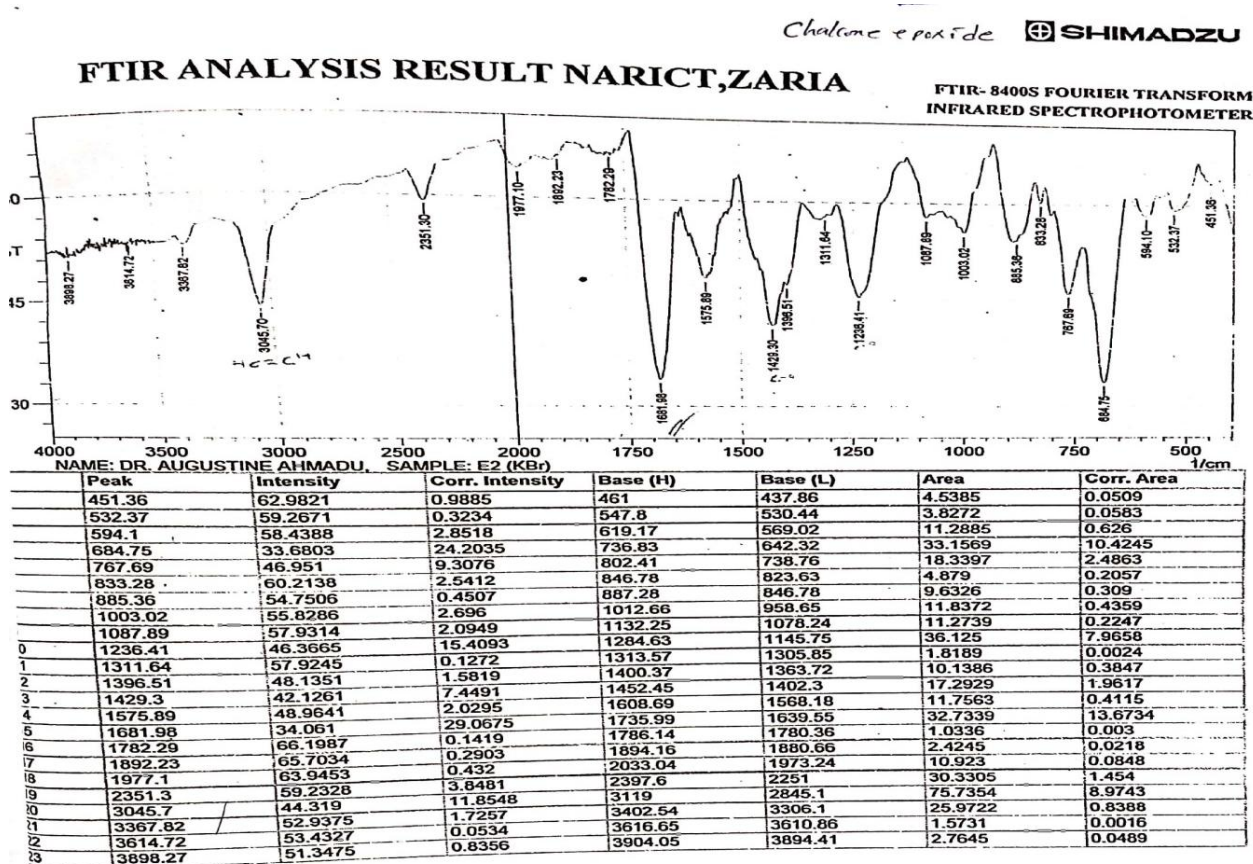


Fig-12: FTIR – Chalcone-epoxide

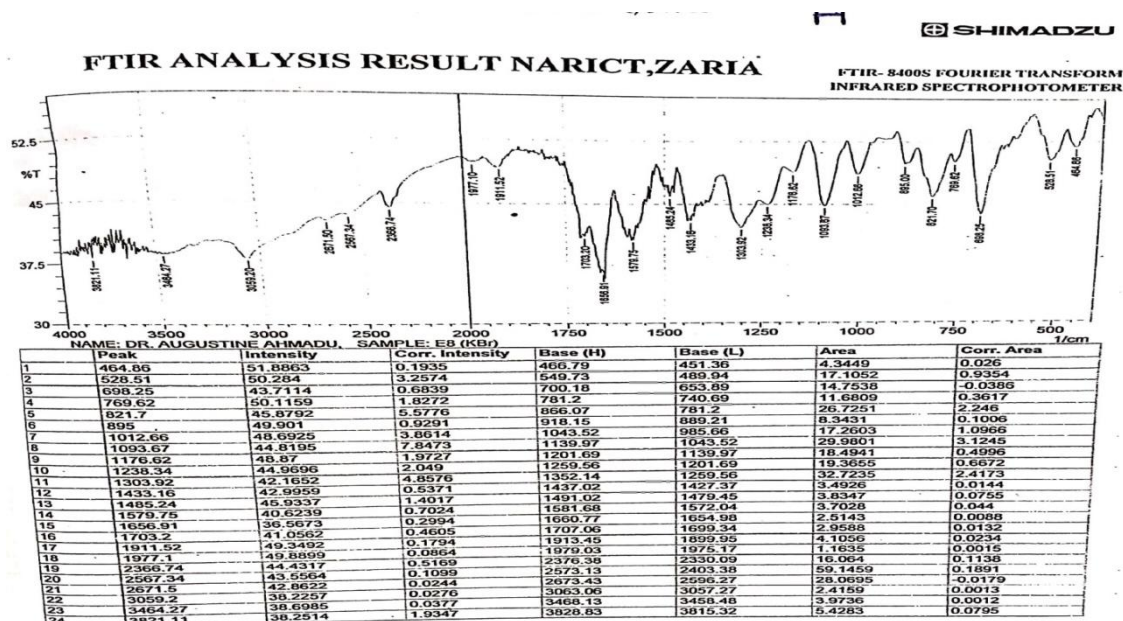


Fig-13: FTIR – 4-Chlorocholeone-epoxide

Table-2: Zone of inhibition of standard drugs used for the microbiological assay

Standard samples	Concentration (mcg/ml)	Zone of inhibition (mm)			
		Mean \pm SD			
		<i>Candida albican</i>	<i>Eserichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Ketoconazole	10,000	10.0 \pm 0.71	0.0 \pm 0.0	0.0 \pm 0.0	5.40 \pm 1.14
	5,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	2,500	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	1,250	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Ciprofloxacin	10	0.0 \pm 0.0	40.0 \pm 1.58	40.0 \pm 1.52	24.4 \pm 1.52
Gentamycin	10	0.0 \pm 0.0	22.8 \pm 2.28	0.0 \pm 0.0	12.4 \pm 1.14
Control (H ₂ O)		0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0

The above shows the zone of inhibition of the standard antimicrobial agents obtained from the microbial bank of the Department of Pharmaceutical

Microbiology and Biotechnology, Niger Delta University.

Table-3: Zone of inhibition of test samples

Test samples	Conc. (mcg/ml)	Zone of inhibition (mm)			
		Mean \pm SD			
		<i>Candida albican</i>	<i>Eserichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Chalcone	10,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	5,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	2,500	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	1,250	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
4-chloro-chalcone	10,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	5,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	2,500	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	1,250	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
Chalcone-epoxide	10,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	5,000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	2,500	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	1,250	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
4-chloro-chalcone epoxide	10,000	24.2 \pm 0.83	0.0 \pm 0.0	0.0 \pm 0.0	13.4 \pm 0.14
	5,000	18.4 \pm 1.14	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	2,500	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	1,250	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0

mcg = microgram, ml = milliliter, mm = millimeter, SD = Standard deviation, Conc. = concentration.

The above shows the zone of inhibition of the synthesized Chalcone and halogenated derivatives at P-value < 0.05. The test p-value was <0.001 from one way ANOVA, which is considered extremely significant. ANOVA assumed that data are sampled from populations with identical standard deviations.

DISCUSSION

In this research, we synthesized chalcone and three substituted derivatives by the scheme illustrated in Figure-1. The test compounds were synthesized in a good percentage of yield and the physical and analytical determination was done by quantifying their various melting points, purified by Thin-Layer Chromatography, and the structural elucidation with the aid of FTIR, ¹HNMR, ¹³CNMR and UV spectrophotometric data. The scheme of the reaction was completed within 24 hours which was ascertained from the TLC single spots for the synthesized compounds. After completion of reaction and workup, the products were identified and characterized using FTIR, ¹HNMR, ¹³CNMR and UV spectrophotometric techniques and their structures were elucidated as chalcone, chalcone-epoxide, 4-chlorochalcone, and 4-chlorochalcone-epoxide respectively.

Antimicrobial screening of the synthesized compounds against different strains of Gram-positive and Gram-negative bacteria and *Candida albican* (fungus). 4-chlorochalcone epoxide showed marked inhibition of both *Staphylococcus aureus* and *Candida albican*. The other compounds were void of both antibacterial and antifungal activity compared with the standard drugs. This is in line with a report on chalcone derivatives by Ere *et al.*, and Awala *et al.*, respectively [12, 17]. Hence this is a (halogenated chalcones) promising lead molecule that can be further modified to achieve a variety of antifungal agents, which are very few in contemporary clinical practice. *Candida albican*, from test results, showed more susceptibility to the synthesized compound (4-chlorochalcone epoxide) than the standard Ketoconazole antifungal agents.

CONCLUSION

This study reveals the level of antimicrobial resistance to antifungal agents by microorganisms and, thus paved the pathway to explore more active and effective antifungal agents using the halogenated chalcones as lead compounds.

Conflict of interest: No conflict of interest

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Authors' Contributions

The study was designed and performed by Dr. Bunu Samuel J, and Pharm. Awala Ebisindor V, while Pharm. Eboh Darlington D. joined in the antimicrobial screening. The article was written by Dr. Bunu Samuel J.

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