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Synthesis of Chalcone and Flavanone Derivatives using ZnO Nanoparticle as Catalyst for Antibacterial Activity

Dinka Mulugeta[1] Bedasa Abdisa[2] Abebe Belay[3] Milkyas Endale[2]*
1.Ethiopian Institute of Agricultural Research (EIAR), P.O.Box 2003, Addis Ababa, Ethiopia
2.Department of Applied Chemistry, Adama Science and Technology University, P.O.Box 1888, Adama, Ethiopia
3.Department of Applied Physics, Adama Science and Technology University, P.O.Box 1888, Adama, Ethiopia

Abstract
A green method was developed for the synthesis of chalcone and flavanone derivatives by Claisen-Schmidth reaction using ZnO nanoparticles as catalyst and water as solvent. The yield of flavonone synthesis reaction catalyzed by ZnO Nanoparticles was found to be higher (89.6%) compared to conventionally protocol (55.7 %) catalyzed by KOH. *In vitro* antibacterial activity of the synthesized compounds against the Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*, *P. aeruginosa* and *K. preumanin* revealed that chalcones **4** and **5** are active on against *S. aureus* (13 and 11 mm zone of inhibition, respectively) whereas flavonones **6** showed promising activity against *E. coli* (10 mm zone of inhibition) and chalcone **5** against *S. aureus and P.aeroginosa*(10 mm zone of inhibition for each) compared to Ceftraxione as positive control.
Keywords: Chalcones, flavanone, Green synthesis, ZnO nanoparticles.

1. Introduction

Chalcones are open chain unsaturated carbonyl system in which two aromatic rings are joined by three carbons having α,β-unsaturated system [1] (Fig 1). Natural chalcones are considered as the precursors of flavonoids and isoflavonoids [3]. Chalcones are popular intermediates for synthesizing various heterocyclic compounds [4] such as flavones, isoxazoles, pyrazoles and tetrahydro-2-chromens [5,6 14,15]. Chalcones have been reported to possess various biological activities such as antimalarial [7], antibacterial [8], anti-cancer [9], antileishmanial [10], antifibrogenic [11] and anti-inflammatory [12] activities.

There were several basic catalysts that have been applied in the synthesis of chalcone, such as NaOH, $Ba(OH)_2$, $Ca(OH)_2$, LiOH, magnesium t-butoxide, potassium carbonate, alumina, MgO, calcinated hydrotalcites. The synthesis reaction has also been carried using acids like $AlCl_3$, dry HCl, $Zn(bpy)(OAC)_2$, $TiCl_4$ and $RuCl_3$. Chalcones have also been synthesized from other methods like using $BF_3.OEt_2$ and Suzuki coupling. However these methods suffer from drawbacks like expensive catalyst, drastic reaction condition, use of toxic and hazardous solvent, longer reaction time, low yield, not applicable to acid and base sensitive functional group, addition of reactant and catalyst in cooling condition, isolation and purification of product requires lot of solvent. Zinc oxide has been employed as a heterogeneous catalyst for various organic transformations. Nano ZnO as heterogeneous catalyst has received considerable attention because of its inexpensive, non-toxic catalyst and has environmental advantages i.e., minimum execution time, low corrosion, waste minimization, recycling of the catalyst, easy transport and disposal of the catalyst. Hence, it is among the suitable candidate to be used as catalyst in chalcone synthesis. Motivated by wide application of nano ZnO to synthetic chemistry, we developed a green approach for the synthesis of chalcone and flavanone derivatives by Claisen-Schmidth reaction using ZnO nanoparticles as catalyst and water as solvent.

2. Experimental

All reagents used in the synthesis were obtained from Sigma-Aldrich Co., St. Louis, MO, USA, and all solvents were purchased from Fine Chemical General Trading PLC, Addis Ababa, Ethiopia. The progress of the reaction was monitored via analytical thin layer chromatograms were run on a readymade 0.2mm thick layer of silica gel GF254 (Merck) coated on aluminum plate *n*-hexane/ethyl acetate as eluent in (7:3) combinations. Column chromatography was performed using silica gel (230-400 mesh) Merck. All the melting points were determined in open capillaries, using digital melting point apparatus, expressed in ^oC. Infrared spectra (FT-IR) were recorded on Perkin Elmer 337 Infrared Spectrophotometer. Samples were screened in Potassium bromide (KBr) pellets and the values are expresses in cm^{-1}. The ^1H and ^{13}C NMR spectra of the compounds were recorded on Bruker avance 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm. Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia.

2.1 Preparation of Zno nanoparticles

ZnO nanoparticles were prepared according to a literature method developed by Pacholski et al. [17] Zinc acetate dihydrated $[Zn(CH_3COO)_2].2H_2O$, 2.4g) and 126 mL of water was added into a round bottom flask. The solution

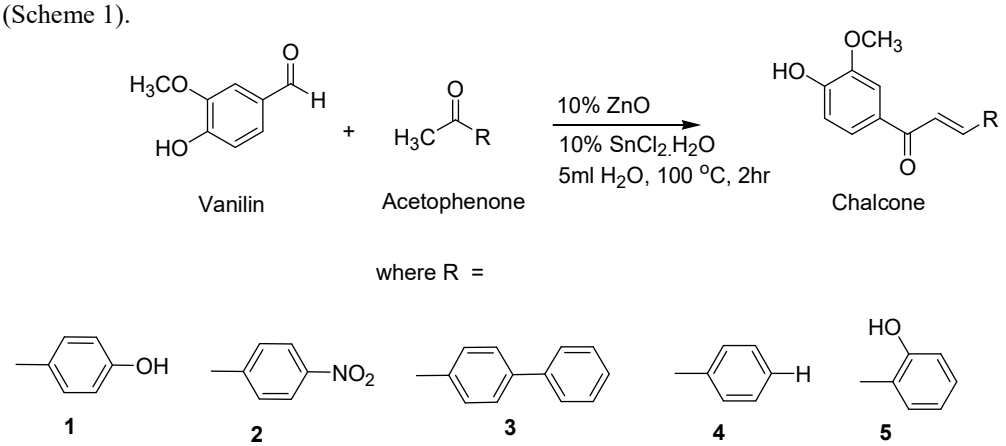
was heated to 60°C with magnetic stirring. The stock solution of potassium hydroxide (KOH) was prepared by dissolving 1.2g of KOH in to 70mL of double distilled water. The stock solution was dropped into the flask within 10-15min. At a constant temperature of 60°C for 2hr. The solution was condensed to about 10-15mL and reheated while stirring for another 5 hr before stopping the heating. The upper fraction of the solution was removed after 30min and water (50mL) was added to the solution and stirred for 5 min. The upper fraction of the solution was discarded again after 30min and dried under vacuum to obtain ZnO nanoparticle.

2.2 General procedure for preparation of chalcones (1-5)

A mixture of equimolar quantities of vanillin and acetophenone derivative (substituted) were mixed with ZnO (0.1mmol.) $SnC1_2.H_2O$ (10 %) and 5mL H_2O and heated at 100°C for 2hr. The progress of the reaction was monitored via TLC using *n*-hexane/ethyl acetate as eluent in (7:3) combinations. The reaction mass was cooled to room temperature and stirred with ethanol (50mL) for 30min and centrifuged for 10 min at 5000rpm. The supernatant was collected and concentrated under reduced pressure. The obtained product was checked by TLC and purified using silica gel column chromatography (230-400 mesh, Merck), with increasing gradient of ethyl acetate in *n*-hexane as the mobile phase. Finally, the product yield was recorded

2.3 General procedure for preparation of flavonones (6-7)

A mixture of equimolar quantities of Chalcone [2'-hydroxychalcone] and Chalcone [(2'-hydroxy-4-nitrochalcone] were mixed with ZnO (0.1 mmol.) $SnC1_2.H_2O$ (10 %) and 5 mL H_2O and heated at 100°C for 2 hr. The progress of the reactions was monitored via TLC using *n*-hexane/ethyl acetate eluent in (7:3) combinations. The reaction mass was cooled to room temperature and stirred with ethanol (50 mL) for 30min and centrifuged for 10 min at 5000 rpm. The supernatant was collected and concentrated under reduced pressure. The obtained product was checked by TLC and purified by silica gel column chromatography (230-400 mesh, Merck), with increasing gradient of ethyl acetate in *n*-hexane as the mobile phase. Finally, the product yield was recorded (Scheme 1).



Scheme 1. Synthesis of chalcones usning ZnO Nps

2.4 Synthesis of flavonone via conventional method

20% of aqueous KOH (2 mmol) was added to a solution of Chalcone [2'-hydroxy-4-nitrochalcone] (4 mmol)) in C_2H_5OH (25 mL). The reaction mixture was stirred at room temperature for 8 hr. The progresses of the reactions were monitored via TLC using *n*-hexane/ethyl acetate eluent in (7:3) combinations. The reaction mass was then poured into ice water and neutralized with aqueous 10% HCl solution The solid residue was recrystallized from ethanol to obtain the purity product.

2.5 Evaluating antibacterial activities

The *in vitro* antimicrobial activity of each compound and the standard drugs was evaluated were using the American Test Culture Collection (ATCC) bacterial strains. Four bacterial strains were obtained from Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. Three Gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Klebsella preumanin* ATCC 35032, Pseudomonas aeruginosa ATCC 9027) and one Gram–positive (Staphylococcus aureus ATCC 25923) human bacterial pathogens were selected for the study. The antibacterial activities were determined using disk diffusion method against different strains of bacteria [16]. Ceftraxione and dimethyl sulfoxide were used as a positive and negative control, respectively.

2.5.1 Media preparation

Mueller Hinton agar was prepared by dissolving the solid media in distilled water. The solution was then

sterilized in autoclave at 121°C for 15 minutes, cooled then poured in Petri dishes. The solution was then left to solidify.

2.5.2 Inoculation and incubation

Antibacterial activity was done on Mueller Hinton agar. 1 mL of bacteria suspension was uniformly spread on the sterile Mueller Hinton Agar Petri dish. Standard solutions of 1.5 mg/mL concentration of the extracts and isolated compounds were prepared and 10 µL solutions from the concentration were loaded to the discs in different replications. 6 mm-diameter wells were cut from the agar using a sterile cork-borer and the sample were placed in the wells. The Petri dish was then placed in an incubator for 24 hours at 37 °C. At the end of incubation period, the inhibition diameter was measured and expressed in millimeters. 3 drops of each bacterial suspension were applied on the Petri dish to compare with 1 drop of dimethyl sulfoxide applied on it. Ceftraxione were used as a positive control while dimethyl sulfoxide was used as a negative control group. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each test organism [16].

3. Results and Discussion

3.1 Yeild Percentage and Physical data

Experimental yield was estimated on the basis of initial weight of the raw materials taken and the final weight of the Chalcone and Flavonones obtained after the complete drying. It was observed that % yield of the reaction in case of ZnO NPS synthesized Flavonone (89.6%) was higher as compared to conventionally synthesized Flavonone (55.7 %) (Table 3). Furthermore effect of $SnCl_2$,H_2O enhancing the cyclization of chalcone by increasing the nucleophilicity of the carbonyl and variation on the % yield of synthesized Flavonone and Reaction time was also evident[17].

The experimental yields and physical data of the synthesized Chalcones (**1-5**) and Flavanones (**6-7**) are listed in Table 1-3.

Table 1. Physical data of chalcones

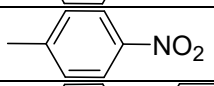
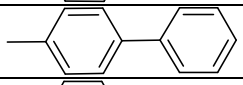
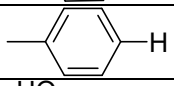
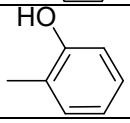
Compound	R	Molecular Formula	Color	Melting point In ^{0}C	Yield %
1		$C_{16}H_{16}O_3$	Pale yellow Solid	89 - 90	80.5
2		$C_{16}H_{13}NO_5$	Yellow Solid	78 - 81	88.9
3		$C_{22}H_{18}O_3$	Yellow Solid	80 -83	85.3
4		$C_{16}H_{15}O_3$	Yellow Solid	86 - 87	80.5
5		$C_{16}H_{16}O_3$	Yellow Solid	79 -82	85.4

Table 2. Physical data of Flavonoes

Compound	R_1 and R_2	Molecular formula	Color	Melting point In ^{0}C	Yield %
6	R_1 = OH , R_2 = OCH_3	$C_{16}H_{14}O_4$	Yellow Solid	89 - 90	89.8
7	R_1= NO_2 , R_2 = H	$C_{15}H_{11}NO_4$	Yellow Solid	87-90	85.5

Table 3**:** Method comparison of % yield of the conventionally and ZnO NPS for synthesis of 2-(4-Nitro-phenyl)-chroman-4-one (**7**)

Method	Time taken for reaction	yield %	Temperature
Conventional	8 hrs	55.7	Room
ZnO NPS	2 hrs	89.6	100 ^{0}C

3.2 Characterization of synthesized compounds

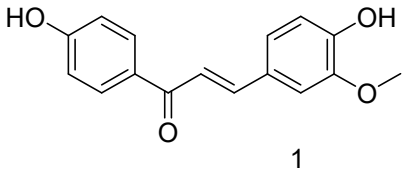
Compound **1** was obtained as Solid Pale yellow (melting point 216-218°C) and its percent yield was 80.5%. The IR (KBr pellet) spectrum showed (ν in cm^{-1}) broad absorption at 3338 cm^{-1} attributed to hydroxyl group. The intense absorption bands at 1651 cm^{-1}, medium bands at 1504 cm^{-1} and intense absorption band at 1170 cm^{-1} showd the presence of carbonyl carbon, aromatic C-C double bond, and the presence of -O-CH_3, respectively.

The ^{1}H NMR spectrum (DMSO-*d*6, 400 MHz) showed a singlet spectra at δ 3.81 (s, 3H) corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern (ring B) was confirmed on the basis of peaks observed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.83 (H-5, *d, J* = 7.2 Hz), δ 7.31 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ7.77(1H, *d*, *J*=15.0Hz), δ7.90 (1H, *d*, *J*=15.0Hz) respectively. The presence aromatic ring with AA'BB' spin system (ring A) was confirmed on the basis of peaks at δ 7.33 (H-2',6', 2H, *dd*, *J*=8.4, 2.1Hz)and δ 6.75 (H-3',5', 2H, *dd*, *J*=7.6, 2.1Hz) (table 4).

The ^{13}C NMR spectrum revealed peak resonating at δ 192.9 attributed to carbonyl carbon. The presence of methoxy group, and carbon-carbon (Cα and Cβ) confimed on the basis of peaks at δ 56.5, 120.8 and 147.9, respectively. The peaks observed at δ 148.7 and 149.7 indicate the presence of two vicinal oxygenated sp2 quaternary carbons on ring B (C-3, C-4) whereas the peak observed at δ 154.7 indicated the presence of sp^2 oxygenated quaternary carbon at C-4' (ring A) (table 4). Thus, on the basis of ^{1}H NMR, ^{13}C NMR and IR spectra data, the structure of compound **1** was established as chalcone 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone.

Table 4. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **1**

Position	**^{1}H NMR**	**^{13}C NMR**
1	-	127.9
2	7.33(1H;d;J=2.0Hz)	116.4
3	-	149.7
4	-	148.7
5	5 6.83(1H;d;*J*=7.2Hz)	116.2
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	132.1
C=O	-	192.9
α	7.77(1H;d;*J*=12.0Hz)	120.8
β	7.90(1H;d;*J*=12.0Hz)	147.9
1'	-	131.1
2'	7.32(1H;d;*J*=8.4Hz)	130.7
3'	6.75(1H;d;J=7,6Hz)	115.9
4'	-	154.2
5'	6.75(1H;;J=7.6Hz)	115.8
6'	7.32(1H;d;J=8.4Hz)	130.7
CH_3O-	3.74(3H; s)	56.5



1

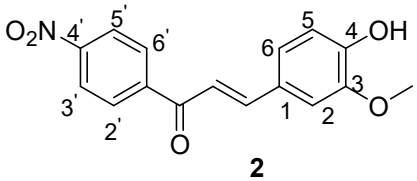
Compound **2** was obtained as yellow solid (melting point 78-81 ºC) and its percent yield was 88.9%. The IR (KBr pellet) spectrum showed (*v* in cm^{-1}) broad absorption at 3338 cm^{-1} attributed to hydroxyl group whereas the presence of carbonyl carbon, aromatic C-C double bond and presence of –O-CH_3 were evident from intense absorption bands observed at 1651 cm^{-1}, 1504 cm^{-1} and 1170 cm^{-1}, respectively

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz) showed a singlet peak which resonates at δ 3.81, corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks at δ 7.33 (H-2, *d*, *J*=2 Hz), δ 6.83 (H-5, *d*; *J* = 7.2, 2.0 Hz), and δ 7.31 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence of *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77(1H, *d*, *J*=15.0Hz) and 7.90 (1H, *d, J*=15.0Hz), respectively. The presence of aromatic ring with AA'BB' spin system was observed at δ 7.33 (H-2',H-6', 2H, *dd, J*=8.4, 2.0Hz) and δ 6.75 (H-3',H-5', 2H, *dd, J*=8.4, 2.0Hz) (table 5).

The ^{13}C NMR spectrum revealed (Table 6) peak at δ 191.5 attributed to carbonyl carbons. The presence of methoxyl group, and carbon-carbon (Cα and Cβ) double bonds were observed at δ 56, 120.8 and 147.9, respectively. The peaks observed at at δ 148.7 (C-4) and 147.7 (C-3) revealed the presence of vicinal sp^2 oxygenated quaternary carbons on ring B whereas the presence of peak at δ 154.7 confirmed the presence of sp^2 carbon connected to nitro group in ring A. Thus, on the basis of the ^{1}H NMR, ^{13}C NMR and IR spectra data of compound (**2**), its structure was established as chalcone 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone.

Table 5. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of Compound **2**

Position	^{1}H NMR	^{13}C NMR
1	-	126.53
2	7.33(1H;d;J=2.0Hz)	115.8
3	-	147.7
4	-	148.9
5	6.83(1H;d;*J*=7.2Hz)	116.2
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	132.1
C=O	-	192.9
α	7.77(1H;d;*J*=15.0Hz)	120
β	No peak on spectra	148
1’	-	131.2
2’	7.32(1H;d;*J*=8.4Hz)	130.8
3’	6.75(1H;d;J=7,6Hz)	115.8
4’	No peak on spectra	153.4
5’	6.75(1H;;J=7.6Hz)	115.7
6’	7.32(1H;d;J=8.4Hz)	130.7
CH_3O-	3.84(3H; s)	56.5



2

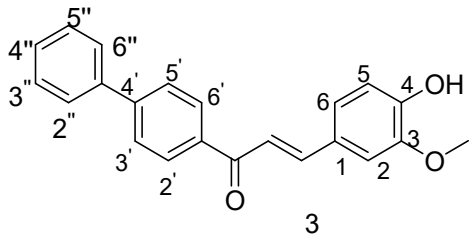
Compound **3** was obtained as yellow solid (melting point 80-83 ^{o}C) and its percent yield was 85.3%. The IR (KBr pellet) spectrum showed (v in cm^{-1}) broad intensity band at 3338 cm^{-1} attributed to hydroxyl group. The intense absorption bands observed at 1651 cm^{-1}, 1504 cm^{-1} and 1170 cm^{-1} suggest the presence of carbonyl carbon, aromatic C-C double bond and presence of –O-CH_3 moiety.

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, table 7) showed a singlet spectra which resonates at δ 3.81, corresponding to a methoxyl group. The presence of aromatic ring with ABX mupltiplicity pattern was confirmed on the basis of peaks observed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.83 (H-5, *d*, *J* = 7.2 Hz) and δ 7.31 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (Hα and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77 (1H, d, *J*=15.0Hz), and δ 7.90 (1H, d, *J*=15.0Hz), respectively. The presence of 1,4 substituted aromatic ring was confirmed on the basis of peaks observed at δ 7.33 (H-2’,6’, 2H, *dd*, *J*=8.4, 2.1Hz), δ 8.2(H-3’,5’, 2H, *dd*, *J*=7,6, 2.1Hz). Additional monosubstituted aromatic ring conneted at C-4’ position of ring A was confirmed on the basis of peaks observed at δ 7.5 (H-2’’,6’’, 2H, *dd J*=8.4, 2Hz), δ 7.5 (H-3’’,5’’, 2H, dd *J*=8.4, 2Hz) and δ 7.4 (H-4’’, 1H, m).

The ^{13}C NMR spectrum showed peak (table 6) at δ 191.5 attributed to carbonyl carbon. The peaks observed at δ 56.3, 120.8 and 147.9 suggest the presence of a methoxyl group and carbon-carbon (Cα and Cβ) double bonds adjacent to carbonyl carbon. The presence of vicinal sp2 oxygenated quaternary carbons on ring B was confirmed from peaks observed at 147.7 and 148.6 (C-3,4) whereas the peak at δ133.7 suggests that a phenyl ring is connected at C-4’ position of ring A (table 6). Thus, on the basis of ^{1}H NMR, ^{13}C NMR and IR spectral data of compound (**3**), its structure was established as chalcone 3-(4-Hydroxy-3-methoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone

Table 6. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **3**

Position	**^{1}H NMR**	**^{13}C NMR**
1	-	126.5
2	7.33(1H;d;J=2.0Hz)	111.1
3	-	148.7
4	-	149.7
5	6.83(1H;d;*J*=7.2Hz)	115.8
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	119.1
C=O	-	191.5
α	7.77(1H;d;*J*=15.0Hz)	124.5
β	No peak on spectra	145.7
1’	-	138.4
2’	7.32(1H;d;*J*=8.4Hz)	129.1
3’	6.75(1H;d;J=7,6Hz)	128.5
4’	No peak on spectra	153.4
5’	6.75(1H;J=7.6Hz)	128.5
6’	7.32(1H;d;J=8.4Hz)	129.1
1”		138.4
2”	7.5(1H;d; *J*=2Hz)	127.3
3”	7.5(1H:dd; *J*=8.4)	129.2
4”	7.4(1H;m; J=1.5Hz)	133.7
5”	7.4(1H;d; J=8.4)	129.2
6”	7.3(1H;d;J=2Hz)	127.4
CH_3O-	3.84(3H; s)	56.3



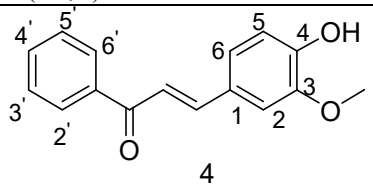
3

Compound **4** was obtained as yellow solid (melting point 86-87ºC) and its percent yield was 80.5%. The IR (KBr pellet) spectrum showed (ν in cm^{-1}) broad absorption peak at at 3338 cm^{-1} attributed to hydroxyl group. The intense absorption bands at 1651 cm^{-1}, 1504 cm^{-1} and 1170 cm^{-1} suggests the presence of carbonyl carbon, aromatic C-C double bond, and O-CH_3 .The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, Appendix 4B) showed a singlet spectra which resonates at δ 3.87, corresponding to a methoxyl group. The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks observed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.83 (H-5, *d*, *J* = 7.2,2 Hz) and δ 7.31 (H-6, *dd*, *J*= 7.2,.0 Hz). The presence *trans* ethylenic protons (αH and Hβ) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77(1H, d, *J*=15.0Hz), δ7.90 (1H, d, *J*=15.0Hz), respectively. The presence of monosubstituted phenyl ring was observed at δ 8.1 (H-2’,H- 6’, 2H, *dd*, *J*=8.4,2Hz), δ 7.7(H-3’,5’, 2H, *dd*, *J*=8.4, 2Hz) and 7.3 (1H, m, H-4’) (table 7).

The ^{13}C NMR spectrum (Appendix 4C) showed peak at δ191.4 attributed to carbonyl carbon. The peaks observed at δ56.2, 124.5 and 145.4 suggests a methoxyl carbon signal and carbon-carbon (C_α and C_β) double bonds adjacent to carbonyl carbon. The two vicinal sp^2 quaternary carbons were observed at δ 147.8 and 148.6 (C-3,4). The peaks observed at δ 138.4 (C-1’), 129.1 (C-2’,6’), 115.9 (C-3’, 5’) and 133.2 (C-4’) suggest the presence of mono substituted aromatic ring (ring A). Thus, based on ^{1}H NMR, ^{13}C NMR and IR spectral data the structure of compound **4** was proposed as shown below (table 7**)**.

Table 7. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **4**

Position	**^{1}H NMR**	**^{13}C NMR**
1	-	128.5
2	7.33(1H;d;J=2.0Hz)	112.1
3	-	149.7
4	-	148.7
5	6.83(1H;d;*J*=7.2Hz)	115.8
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	119.1
C=O	-	191.4
α	7.77(1H;d;*J*=15.0Hz)	124.5
β	7.90(1H;d;*J*=15.0Hz)	145.4
1’	-	138..4
2’	7.32(1H;d;*J*=8.4Hz)	129.1
3’	6.75(1H;d;J=7.6Hz)	115.9
4’	8.3(1H;m;J=1.5Hz)	133.2
5’	6.75(1H;;J=7.6Hz)	115.8
6’	7.32(1H;d;J=8.4Hz)	129.1
CH_3O-	3.74(3H; s)	56.2



4

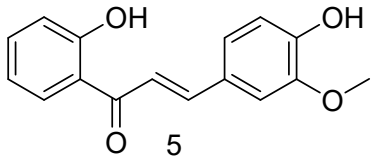
Compound **5** was obtained as yellow solid (melting point 79-82 ^{0}C) and its percent yield was 85.4%. The IR (KBr pellet) spectrum showed (ν in cm^{-1}) broad absorption band at 3338 cm^{-1} attributed to hydroxyl group.The intense absorption bands at 1651 cm^{-1}, 1504 cm^{-1}, and 1170 cm^{-1} suggest the presence of carbonyl carbon, aromatic C-C double bond, and O-CH_3 moiety .

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz) showed a singlet spectra which resonates at δ 3.87, corresponding to a methoxy group. The presence of aromatic ring with ABX multiplicity pattern was confirmed at δ 7.33 (H-2, *d*, *J*= 2 Hz), δ 6.87 (H-5, *d*; *J* = 7.2 Hz) and δ 6.98 (H-6, *dd*, *J* = 7.2, 2.0 Hz). The presence *trans* ethylenic protons (αH and βH) adjacent to the carbonyl were evident from ^{1}H NMR spectrum δ 7.77(1H, d, *J*=15.0Hz) and 8.2 (1H, d, *J*=15.0Hz), respectively . The presence of another disubstituted aromatic ring (ring A) was confirmed on the basis of peaks observed at δ7.8(H-6’,1H, *dd*, *J*=8.4, 2Hz), δ7.5 (H-4’,1H, *dd*, *J*=;8.4, 2Hz), δ7.3 (H-3’,1H, *dd*, *J*=8.4, 2Hz) and δ6.9 (H-5’,1H, *dd*, *J*=;8.4, 2Hz) (table 8).

The ^{13}C NMR spectrum of showed peak at δ194.1 attributed to carbonyl carbon. The presence of peaks at δ56.3, 126.5 and 146.6 ppm suggests the presence of methoxyl carbon signal, and carbon-carbon (C_α and C_β) double bonds adjacent to carbonyl. The presence of sp^2 oxygenated quaternary carbon at at δ 162.6 (C-2’) confirms the presence of hydroxyl group at C-2’ position. Thus, based on the IR, ^{1}H NMR and ^{13}C NMR spectral data the following structure was suggested for compound **5**.

Table 8. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **5**

Position	**^{1}H NMR**	**^{13}C NMR**
1	-	126.5
2	7.33(1H;d;J=2.0Hz)	116.4
3	-	140.8
4	-	148.7
5	6.83(1H;d;*J*=7.2Hz)	116.2
6	7.31(1H;dd;*J*=7.2 , 2.0Hz)	119.5
C=O	-	192.9
α	7.77(1H;d;*J*=15.0Hz)	120.9
β	7.90(1H;d;*J*=15.0Hz)	146.6
1’	-	125.3
2’	7.32(1H;d;*J*=8.4Hz)	131.1
3’	7.3(1H;dd;J=8.4Hz,2Hz)	120.9
4’	7.5(1H;dd;J=8.4Hz,2Hz)	136.6
5’	6.9(1H;dd;J=8.4Hz,2Hz)	116.1
6’	7.8(1H;dd;J=8.4Hz,2Hz)	162.6
CH_3O-	3.74(3H; s)	56.5



5

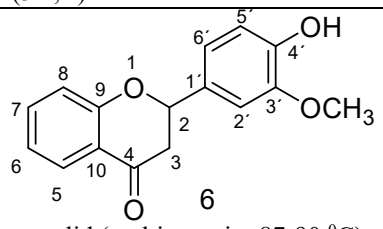
Compound **6** was obtained as yellow solid (melting point 80-89 ^{0}C) and its percent yield was 89.8%. The IR (KBr pellet) spectrum showed (v in cm^{-1}): broad absorption at 3043 cm^{-1} attributed to hydroxyl group. The intense absorption bands observed at 1706 cm^{-1}, 1589 cm^{-1}, 1170 cm^{-1} and 1103 cm^{-1} suggest the presence of carbonyl carbon, aromatic C-C double bond, O-CH_3, and –C-O-C- group moiety.

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, table10) showed a singlet spectra which resonates at δ 3.87, corresponding to a methoxyl group. The presence of oxygenated sp3 methine at δ 5.35 ((H-2, dd; J = 2.79 and J =12.31) and methylene peak at δ 2.70 (H-3, *dd*; J =2.79, 16.72). The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks observed at δ 6.76 (H-5’, d, J = 8.9), δ 6.83(H-6’, d, J = 8.82) and δ 6.96(H-2’, d, J=2Hz). The presence of disubstituted aromatic ring (ring A) was confirmed δ 6.90 (H-7,*dd*, *J*=2.79, 8Hz), δ 7.27 (H-5, *dd*, J = 8.9, 2Hz), δ6.60(H-6, *dd*,J=8, 2Hz), and δ6.8(H-6, *dd*, J=8, 2Hz).

The ^{13}C NMR spectrum showed peaks at 181.8 and 56.5attributed to carbonyl carbon and methoxyl carbon. The presence of sp3 oxygenated methine and methylene alpha to carbonyl carbon was confirmed at δ78.3 (C-2) and δ60 (C-3). The presence of peaks at δ147.7 and 148.1 confirm the presence of vicinal sp^2 oxygenated quaternary carbons (C-3’, 4’). Thus, based on the above spectral data the structure of compound **6** was suggested as shown below.

Table 9. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **6**

Position	**^{1}H NMR**	**^{13}C NMR**
1	-	-
2	5.33(1H;dd;J=2.79Hz,16.72Hz)	78.3
3	2.70(IH;dd;J=2.79Hz,16.72Hz	60.3
4	-	181.8
5	7.27(1H,d,*J*=8.94Hz	120.7
6	6.60(1H,d,J=8.82Hz)	112.3
7	6.90(1H; dd, J=2.79, 8Hz)	136.6
8	6.8(1H,d,J=8.94Hz,)	114.9
1’	-	158.7
2’	6.96(1;d;J=1.86Hz)	116.3
3’		147.7
4’		148..1
5’	6.76(1H;d;J=8.13,)	116.3
6’	6.83(1H;dd;J=1.86,J=8.13)	120.7
CH_3O-	3.74(3H; s)	56.5



6

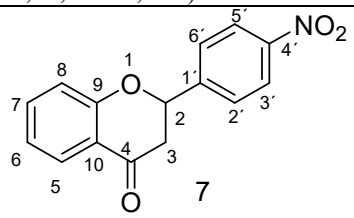
Compound **7** was obtained as yellow solid (melting point 87-90 ^{0}C) and its percent yield was 85.0%. The IR (KBr pellet) spectrum showed (v in cm^{-1}): broad absorption at 3043 cm^{-1} attributed to hydroxyl group. The intense absorption bands observed at 1706 cm^{-1}, 1650 cm^{-1}, 1589 cm^{-1}, 1170 cm^{-1} and 1103 cm^{-1} suggest the presence of carbonyl carbon, NO_2, aromatic C-C double bond, O-CH_3, and –C-O-C- group moiety.

The ^{1}H NMR spectrum (DMSO-d_6, 400 MHz, table10) showed presence of oxygenated sp^3 methine at δ 5.35 ((H-2, dd; J = 2.79 and J =12.31) and methylene peak at δ 2.50 (H-3, *dd*; J =2.79, 16.72). The presence of aromatic ring with ABX multiplicity pattern was confirmed on the basis of peaks observed at δ 8.12 (H-5’, d, J= 8.9), δ 7.45(H-6’, d, J = 8.82) and δ 7.12(H-2’, d, J=2Hz). The presence of disubstituted aromatic ring (ring A) was confirmed δ 6.90 (H-7,*dd*, J=2.79, 8Hz), δ 7.27 (H-5, *dd*, J = 8.9, 2Hz), δ6.60(H-6, *dd*, J=8, 2Hz), and δ6.8(H-6, *dd*, J=8, 2Hz).

The ^{13}C NMR spectrum showed peaks at δ 191.4 attributed to carbonyl carbon The presence of sp^3 oxygenated methine and methylene alpha to carbonyl carbon was confirmed at δ 78.3 (C-2) and δ 60 (C-3). The presence of peaks at δ 147.7 and 148.1 confirm the presence of quaternary carbons (C-1’, 4’). Thus, based on the above spectral data the structure of compound **7** was confirmed as shown below

Table 10. ^{1}H NMR ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **7**

Position	^{1}H NMR	^{13}C NMR
1	-	-
2	5.35(1H;dd;J=2.79Hz,16.72Hz)	78.3
3	2.50(IH;dd;J=2.79Hz.16.72Hz	60.3
4	-	191.3
5	7.27(1H,dd,*J*=8.9Hz,2Hz	120.7
6	76.6(1H,dd,J=8.8HZ, 2Hz)	121.1
7	6.90(1H, dd, J=2.79Hz,12.31Hz)	136.6
8	7.27(1H,d,J=8.94Hz,)	118.5
9		161.1
1’	-	128.1
2’	7.12(1H;d;J=2Hz)	124.2
3’	6.76(1H;d;J=8.13,)	122.1
4’		148..1
5’	8.12(1H;d;J=8.9)	124.2
6’	7.45(1H;dd;J=8Hz,2Hz)	129.1



7

3.2 Antibacterial Activity

All the chalcones and flavonones **(1-7)** have been evaluated for their antibacterial activity against *S. aureus* **(**Gram-positive) and *E. coli, K. pneumonia* and *P. aeroginosa* (Gram-negative) using disk diffusion method. The results of this evaluation were compared with cefraxone as reference standard (table 11). It is interesting to note from the results that almost all the compounds exhibited some degree of inhibition zones. The chalcones **4** and **5** are active on against *S. aureus* were as Flavonones **6** active on against *E. coli* and **7** active on against *S. aureus* and *P. aeroginosa* (Figure 1).

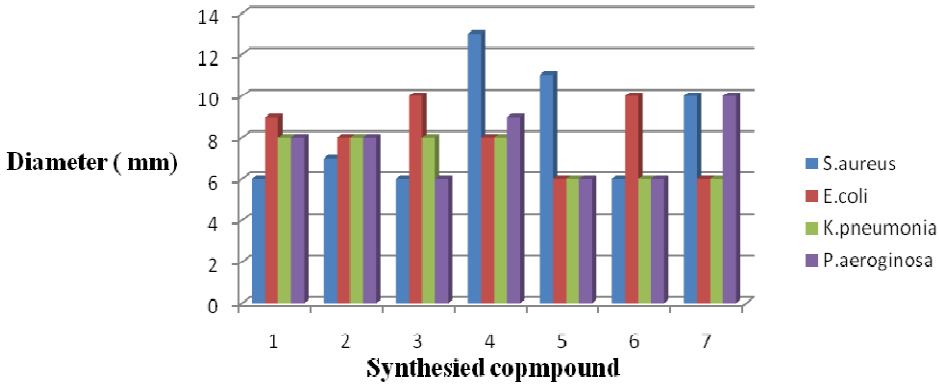


Fig. 1. Diameters of ihnibition of test Compounds on test microbes

4. Conclusion

The study explored the scope of application of ZnO nanoparticle as a catalyst for synthesis of chalcone derivatives of vanillin and different substituted acetophenone derivatives with electron donating and withdrawing groups at para position of acetophenone. The Claisen-Schmidt condensation reaction proceeded smoothly to furnish chalcones and flavanones derivatives in good yield. The study reported inexpensive and easily available solid base catalyst, shorter reaction time, high yield, easy work up procedure and no side product compared to conventional method using potassium hydroxide. The method can also be applied to synthesis of chalcone derivatives comprising base sensitive functional groups with high yield, short reaction time and easy work up procedure**.** Use of $SnCl_2$,H_2O enhance the cyclization of chalcone to flavanones which was evidenced

by rapid increase in the yield of synthesized flavonone and reaction time. *In vitro* antibacterial activity of the synthesized compounds against the Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli*, *P. aeruginosa* and *K. preumanin* revealed that chalcones **4** and **5** are active on against *S. aureus* (13 and 11 mm zone of inhibition, respectively) whereas flavonones **6** showed promising activity against *E. coli* (10mm zone of inhibition) and chalcone **5** against *S. aureus* and *P. aeroginosa*(10mm zone of inhibition for each) compared to Ceftraxione as positive control.

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