CHAPTER II

EXPERIMENTAL

2.1 Chemical, apparatus and instruments

2.1.1 Chemicals

The chemicals used in this research project were as listed in Table 2

Table 2 The chemicals used in this research

Chemical	Molecular	Molecular weight	Grade	Supplier
Acetone*	C_3H_6O	58.08	commercial	Labscan
Ally amine	C_3H_7N	57.10	≥ 99.0 %	Fluka
4-Amino-pyridin	$C_5H_6N_2$	94.12	≥ 98.0 %	Fluka
Benzophenone	$C_{13}H_{10}O$	182.22	≥ 99.0 %	Fluka
2-Bromobenzaldehyde	C_7H_5OBr	185.03	≥ 97.0 %	Fluka
Calcium hydride	CaH_2	42.10	≥ 97.0 %	Fluka
Dichloromethane*	CH_2Cl_2	84.93	commercial	Labscan
Diethyl ether*	$C_4H_{10}O$	74.12	commercial	Labscan
95% Ethanol*	C_2H_6O	46.06	commercial	Labscan
Ethyl acetate*	$C_4H_8O_2$	88.11	≥ 99.0 %	Labscan
Hexane*	C_6H_{14}	86.10	commercial	Labscan
Hydrochloric acid	HCl	36.50	≥ 35.0 %	Merck
Hydroxylamine hydrochloride	$NH_2OH.HCl$	69.49	≥ 99.0 %	Aldrich
Lithium aluminium hydride	$LiAlH_4$	37.95	≥ 97.0 %	Merck
Oxalyl chloride	$C_2O_2Cl_2$	126.93	≥ 99.0 %	Merck
Potassium Hydroxide	KOH	56.10	≥ 85.0 %	Fluka

Table 2 (continued)

Chemical	Molecular	Molecular weight	Grade	Supplier
Sodium chloride	NaCl	58.44	-	TRS
Sodium hydroxide	NaOH	40.00	≥ 99.0 %	Thasco
Sodium metal	Na	22.99	-	-
Sodium sulfate anhydrous	Na_2SO_4	142.04	≥ 99.0 %	BDH
Tetrahydrofuran**	C_4H_8O	72.11	≥ 99.0 %	Fluka
Triethylamine***	$C_6H_{15}N$	101.19	≥ 99.0 %	Scharlau
Silica gel 60 PF254	-	-	-	Scharlau
Silica gel 60 GE0030	-	-	-	Fluka

Note * Simple distillation

** Distilled from sodium / benzophenone under nitrogen atmosphere

*** Refluxed over CaH_2 for 2 h followed by simple distillation

2.1.2 Apparatus and instruments

The apparatus and instruments used were listed in Table 3

Table 3 The apparatus and instruments used in this research

Apparatus and instruments	Company	Model
High vacuum pump	Edwards	Edwards 18
Infrared Spectrometer (FT-IR)	Bruker	Tensor 27
Mass spectrometer (HRMS)	Waters	Micromass Q-Tof-2^{Tm}
Melting point apparatus	Sunyo	Galle Kamp
Nuclear Magnetic Resonance Spectrometer	Bruker	DRX 400
Rotary evaporator	Büchi	R-200
UV-lamp254	Vilber Lourmat	-
Weighing balance (4 positions)	Toledo	AB204-S

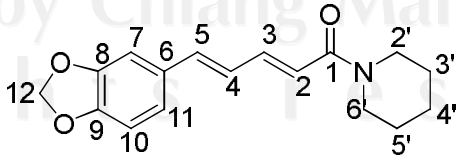
2.2 Experimental procedures

2.2.1 Isolation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid piperidine amide (piperine) (1) from black pepper[32,33]

Dried fruits of *Piper nigrum* Linn were purchased from the local market in Chiang Mai province. The black pepper dried fruits were collected and air-dried. Then the dried fruits were ground into a powder.

The powder fruits material was extracted with 95 % ethanol and refluxed 24 h, filtered and concentrated by rotary evaporator until obtained a dark brown viscous liquid. Alcoholic potassium hydroxide 10 % was added to precipitate the residue, filtered and the filtrate left for one day to precipitate the crude piperine. The crude piperine was purified by crystallization from dichloromethane: hexane (3:2) to give the pure piperine (1.13 % yield) as yellow crystals from CH_2Cl_2/hexane, m.p.130.8-132.0 ºC.

The structure of piperine was characterized by ^{1}H NMR and MS spectral data. The conditions for isolation of piperine and the spectroscopic data of piperine were shown in Table 4.

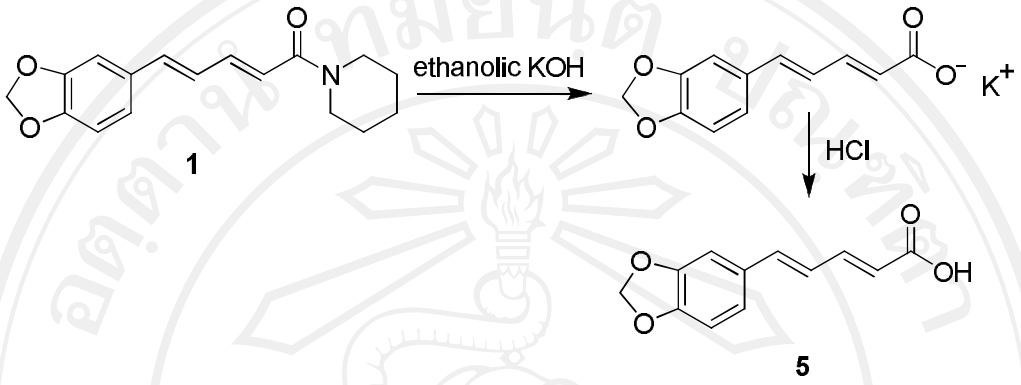


1

Table 4 Data of piperine (**1**)

Physical properties	
Yellow crystals m.p.130.8-132.0 ºC (CH_2Cl_2/hexane) (Lit.[34] 131-134 ºC)	
IR Spectroscopy (KBr-pellet)	
Frequency (cm^{-1})	Type of vibrations
2930	C-H stretch of CH_2
1680	C=O stretch of 3°-amide
1630	C=C stretch of the conjugated double bonds
1600	C=C stretch of a benzene ring
1250	C-N stretch
1000	C-O stretch
NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
1.45-1.74	6H, *m*, H-3′,4′,5′
3.60	4H, *br*, H-2′,6′
6.00	2H, *s*, H-12
6.51	1H, *d* (J = 14.6 Hz), H-2
6.67-6.80	3H, *m*, H-5,10, 11
6.90	1H, *dd* (J = 1.5, 8.0 Hz), H-4
7.00	1H, *s*, H-7
7.38-7.41	1H, *m*, H-3

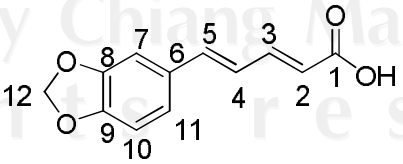
2.2.2 General procedure for preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E -pentadienoic acid (piperic acid) (5) from piperine (1)[32,33]



1 5

Piperine (2.8536 g, 10 mmol) was refluxed with ethanolic KOH (2 N, 10 ml) for 25 h. Ethanol was removed off by rotary evaporator to obtain the solid potassium salt of piperic acid, then follow by dissolved in hot water 50 ml, acidified with 35 % HCl to give the yellow precipitate and recrystallization with ethanol to obtain piperic acid (**5**) in 98.98 % yield as yellow needles.

The structure of piperic acid (**5**) was characterized by ^{1}H NMR, IR and MS. The reaction conditions for alkaline hydrolysis of piperic acid from piperine and the spectroscopic data of piperic acid were shown in Table 5.



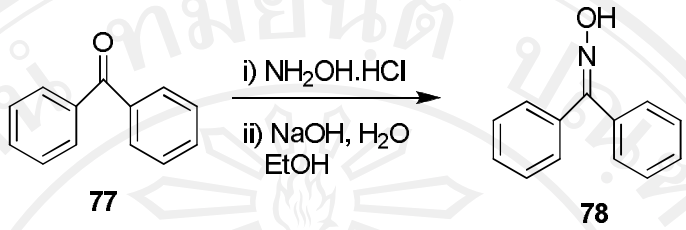
5

Table 5 Data of piperic acid (**5**)

Physical properties	
Yellow needles m.p. 217.0-217.8 ºC (EtOH) (Lit.[24] 217.8-218.5 ºC)	
IR Spectroscopy (KBr-pellet)	
Frequency (cm^{-1})	Type of vibrations
3300-2400 (br)	O-H stretch of hydroxyl group
3050, 3100	C-H stretch of benzene ring
2950, 2830	C-H stretch of CH_2
1690	C=O stretch
1630	C=C stretch of the conjugated double bonds
1600, 1500	C=C stretch of the benzene ring
1050, 1250	C-O stretch
NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
5.92	1H, *d* (*J* = 15.1 Hz), H-2
6.05	2H, *s*, H-12
6.92	1H, *d* (*J* = 8.0 Hz), H-5
6.96	1H, *s*, H-7
6.97	1H, *d* (*J* = 3.5 Hz), H-11
7.00	1H, *dd* (*J* = 1.5, 8.0 Hz), H-4
7.23	1H, *d* (*J* = 1.5 Hz), H-10
7.25-7.32	1H, *m*, H-3
12.19	1H, *br,* OH

2.2.3 General procedure for preparation of oxime[35,36]

2.2.3.1 Preparation of benzophenone oxime (78)

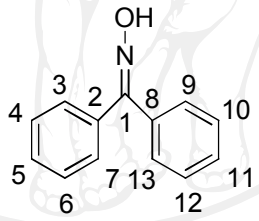


Three conditions were used to study the preparation of benzophenone oxime. A mixture of benzophenone (**77**), sodium hydroxide and hydroxylamine hydrochloride in EtOH (50 mL) was stirred at reflux for 3-6 h. After cooling, the mixture was neutralized and extracted by CH_2Cl_2 (3x100 mL). The organic layer was dried over Na_2SO_4. The reaction mixture was evaporated under reduced pressure to give the white crude product and then purified by column chromatography (silica gel; ethyl acetate in hexane, 1:9, as eluent) to obtain compound 78 (70.32-99.02 % yield) as white crystals from CH_2Cl_2/hexane, m.p. 140.8-144.4 ºC, 72-100 % conversion from the starting material.

The structure of benzophenone oxime **78** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 6 and 7.

Table 6 The various reaction conditions used for the preparation of compound **78**

Entry	Conditions	Time (h)	% yield of 78
1	Benzophenone (1.0000 g, 5.5 mmol) $NH_2OH.HCl$ (0.5727 g, 8.2 mmol) NaOH (0.3283 g, 8.2 mmol)	3	70.32 % (0.7611 g)
2	Benzophenone (1.0004 g, 5.5 mmol) $NH_2OH.HCl$ (1.1445 g, 16.5 mmol) NaOH (1.3170 g, 32.9 mmol)	3	97.65 % (1.0574 g)
3	Benzophenone (1.0000 g, 5.5 mmol) $NH_2OH.HCl$ (1.1448 g, 16.5 mmol) NaOH (1.3173 g, 32.9 mmol)	6	99.02 % (1.0718 g)



78

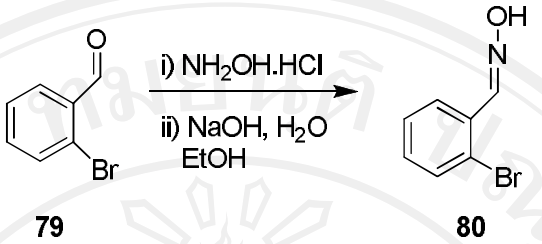
Table 7 Data of benzophenone oxime (**78**)

Physical properties	
White crystals m.p. 140.8-144.4 ºC (CH_2Cl_2/hexane) (Lit.[37] 139-142 ºC)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3430 (br)	O-H stretch of hydroxyl group
1632	C=N stretch
1327	C=C stretch of the benzene ring
1156	C-O stretch

Table 7 (continued)

Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{13}H_{11}NO$	197.0919 $(M)^+$
Calc. mass for $C_{13}H_9N$	180.7138 $(M\text{-}H_2O)^+$
Found for $C_{13}H_9N$	180.7152 $(M\text{-}H_2O)^+$
NMR spectroscopy	
^1H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
7.31-7.41	3H, *m*, H-4,5,6
7.41-7.43	2H, *m*, H-3,7
7.45-7.50	5H, *m*, H-9,10,11,12,13
9.10	1H, *s,* OH
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
127.51, 127.84, 128.22, 128.32, 128.87, 129.09, 129.19, 129.21, 129.49, 128.81	CH-3,4,5,6,7,9,10,11,12,13
132.64	C_q-2
136.17	C_q-8
157.93	C=N-1

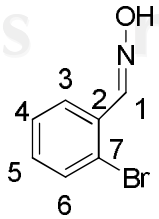
2.2.3.2 Preparation of 2-bromobenzaldehyde oxime (80)



79 **80**

A mixture of 2-bromobenzaldehyde (1.5850 g, 8.6 mmol), sodium hydroxide (1.9869 g, 49.7 mmol) and hydroxylamine hydrochloride (1.8048 g, 26.0 mmol) in EtOH (50 mL) was stirred at reflux for 3 h. After cooling, the mixture was neutralized and extracted by CH_2Cl_2 (3x100 mL). The organic layer was dried over Na_2SO_4. The reaction mixture was evaporated under vacuum pressure to give the light yellow crude product and then purified by column chromatography (silica gel; ethyl acetate in hexane, 1:9, as eluent) to obtain compound **80** (1.4370 g, 83.78 % yield) as white crystals from CH_2Cl_2/hexane, m.p. 88.8-91.1 ºC, 96 % conversion from the starting material.

The structure of 2-bromobenzaldehyde oxime **80** was characterized by ^1H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 8.



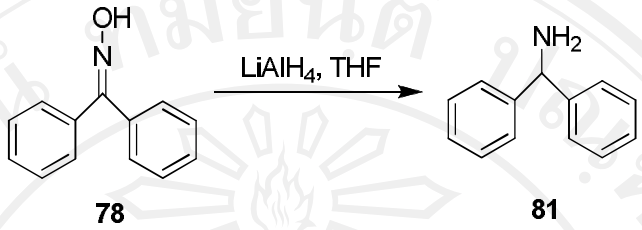
80

Table 8 Data of 2-bromobenzaldehyde oxime (**80**)

Physical properties	
White crystals m.p. 88.8-91.1 ºC (CH_2Cl_2/hexane) (Lit.[38] 88-90 ºC)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3396 (br) 1642 748,971	O-H stretch of hydroxyl group C=N stretch C-Br stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for C_7H_6BrNO Calc. mass for C_7H_7BrNO Found for C_7H_7BrNO	198.9633 $(M)^+$ 199.9711 $(M+H)^+$ 199.9721 $(M+H)^+$
NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (*δ,* ppm)	Type of protons
7.20-7.25 7.32 7.57 7.79 8.58 10.36	1H, *m*, H-5 1H, *t* (*J* = 7.42 Hz), H-4 1H, *dd* (*J* = 7.98, 1.19 Hz), H-6 1H, *dd* (*J* = 7.79 Hz), H-3 1H, *s*, H-1 1H, *s,* OH
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (*δ,* ppm)	Type of carbons
123.84 127.46 127.60 131.21 131.36 133.17 149.82	C_q-7 CH-3 CH-4 CH-5 CH-6 C_q-2 CH-1

2.2.4 General procedure for preparation of amines[35]

2.2.4.1 Preparation of diphenylmethanamine (81)



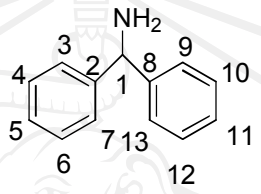
78 81

Two conditions were used to study the preparation of diphenylmethanamine. Benzophenone oxime (**78**), in anhydrous THF (10 mL), was added dropwise to $LiAlH_4$ in anhydrous THF (10 mL). The mixture was stirred under reflux for 3-6 h. After cooling, the mixture was hydrolyzed and extracted by CH_2Cl_2 (3x100 mL). The organic layer was dried over Na_2SO_4. The reaction mixture was evaporated under vacuum pressure to give the brown liquid product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:8, as eluent) to obtain compound **81** (29.33-34.47 % yield) as colorless liquid, 46-52 % conversion from the starting material.

The structure of diphenylmethanamine **81** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 9 and 10.

Table 9 The various reaction conditions used for the preparation of compound **81**

Entry	Conditions	Time (h)	% yield of 81
1	Benzophenone oxime (0.8001 g, 4.1 mmol) $LiAlH_4$ (0.6177 g, 16.3 mmol)	3	29.33 % (0.2180 g)
2	Benzophenone oxime (0.8000 g, 4.1 mmol) $LiAlH_4$ (0.6172 g, 16.3 mmol)	6	34.47 % (0.2562 g)



81

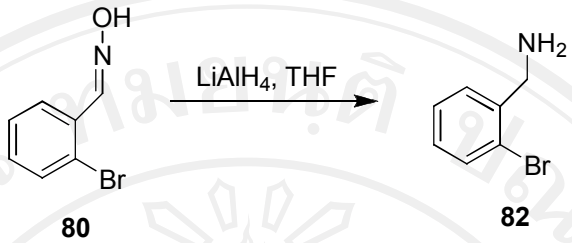
Table 10 Data of diphenylmethanamine (**81**)

Physical properties	
Colorless liquid	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3373,3300	N-H stretch of primary amine group
3026	C-H stretch of CH
1600, 1492	C=C stretch of the benzene ring
1027	C-N stretch

Table 10 (continued)

Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{13}H_{13}N$	183.1048 $(M)^+$
Calc. mass for $C_{13}H_{13}NNa$	206.0946 $(M+Na)^+$
Found for $C_{13}H_{13}NNa$	167.0861 $(M-NH_3)^+$
NMR spectroscopy	
^1H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
5.25	1H, *s*, H-1
7.25-7.28	2H, *m*, H-5,11
7.32-7.37	4H, *m*, H-4,6,10,12
7.41-7.47	4H, *m*, H-3,7,9,13
1.86	2H, *s*, NH_2
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
59.67	CH-1
126.83	CH-5,11
126.88	CH-4,6,10,12
128.40	CH-3,7,9,13
145.55	C_q-2,8

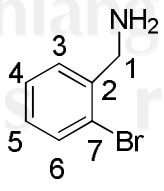
2.2.4.2 Preparation of 2-bromobenzylamine (82)



80 **82**

2-bromobenzaldehyde oxime (0.8000 g, 4.0 mmol) in anhydrous THF (10 mL) was added dropwise to $LiAlH_4$ (0.6060 g, 16.0 mmol) in anhydrous THF (10 mL). The mixture was stirred under reflux for 6 h. After cooling, the mixture was hydrolyzed and extracted by CH_2Cl_2 (3x100 mL). The organic layer was dried over Na_2SO_4. The reaction mixture was evaporated under vacuum pressure to give the grey liquid product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:8, as eluent) to obtain compound **82** (0.2362 g, 31.74 % yield) as slightly yellow liquid, 48 % conversion from the starting material.

The structure of 2-bromobenzylamine **82** was characterized by ^1H NMR, NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 11.



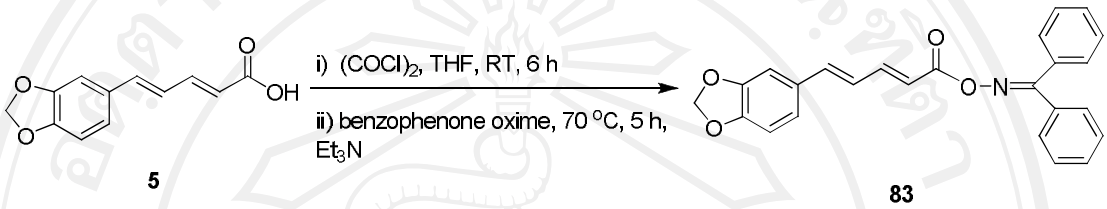
82

Table 11 Data of 2-bromobenzylamine (**82**)

Physical properties	
Slightly yellow liquid	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
33,623,400	N-H stretch of primary amine group
3030	C-H stretch of CH
1569	C=C stretch of the benzene ring
1324	C-N stretch
698,738	C-Br stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for C_7H_8BrN	184.9840 $(M)^+$
Calc. mass for C_7H_6BrNNa	207.9581 $(M+Na)^+$
Found for C_7H_6BrN	205.9592 $(M+Na-H_2)^+$
NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
5.34	2H, *s*, H-1
7.25	2H, *m*, H-3,5
7.37	1H, *m*, H-4
7.78	1H, *m*, H-6
2.03	2H, *s*, NH_2

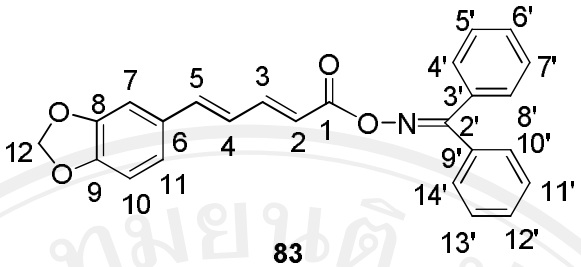
2.2.5 General procedure for preparation of amides and oxime-ester derivatives of piperine[32,33]

2.2.5.1 Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid benzophenone oxime ester (83)



To a solution of piperic acid (0.5001 g, 2.3 mmol) in dried THF (4 ml) at room temperature under nitrogen atmosphere was added oxalyl chloride (1 ml, 11.5 mmol) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride as an orange residue. To the crude piperic acid chloride in dried THF (4 ml), was added the solution of benzophenone oxime (0.4523 g, 2.3 mmol) in dried THF (3 ml) followed by triethylamine (0.48 ml, 3.4 mmol) and stirred at 70 ºC for 5 h. The reaction mixture was evaporated to give the yellow crude product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 1:9, as eluent) to obtain compound **83** (0.2562 g, 28.13 % yield) as yellow crystals from CH_2Cl_2/hexane, m.p. 207.3-208.1 ºC, 75 % conversion from the starting material.

The structure of compound **83** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 12.



83

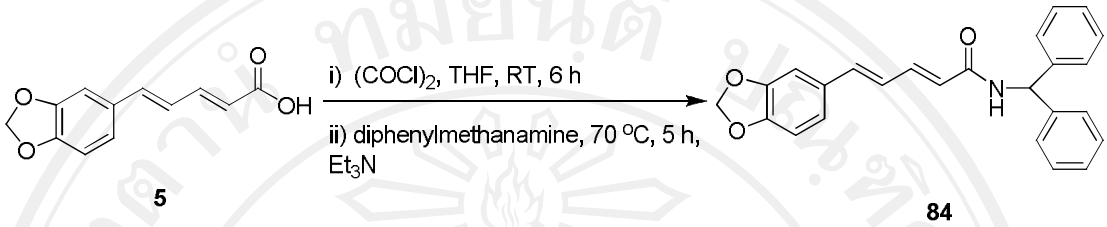
Table 12 Data of compound **83**

Physical properties	
Yellow crystals m.p. 207.3-208.1 ºC (CH_2Cl_2/hexane)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3050, 3100	C-H stretch of benzene ring
2950, 2830	C-H stretch of CH_2
1690	C=O stretch
1600, 1500	C=C stretch of the benzene ring
1325	C-N stretch
1050, 1250	C-O stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{25}H_{19}NO_4$	397.1314 $(M)^+$
Calc. mass for $C_{25}H_{19}NO_4Na$	420.1212 $(M+Na)^+$
Found for $C_{25}H_{19}NO_4Na$	420.1210 $(M+Na)^+$

Table 12 (continued)

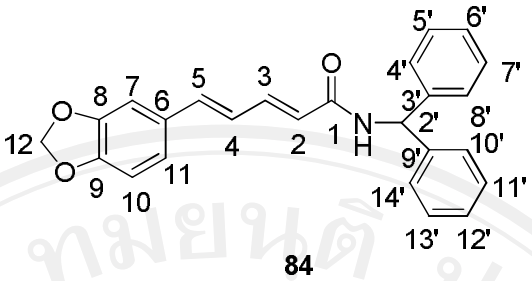
NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
5.89	1H, *d* (*J* = 15.26 Hz), H-2
5.97	2H, *s*, H-12
6.62-6.70	1H, *m*, H-4
6.73-6.78	2H, *m*, H-5,10
6.89	1H, *dd* (*J* = 8.06, 1.58 Hz), H-11
6.95	1H, *d* (*J* = 1.49 Hz), H-7
7.32-7.38, 7.46-7.50, 7.59-7.64	10H, *m*, H-4′,5′,6′,7′,8′,10′,11′,12′,13′,14′
7.40-7.46	1H, *m*, H-3
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
101.35	CH_2-12
105.80	CH-7
108.48	CH-10
117.67	CH-2
123.11	CH-11
124.36	CH-4
128.12	CH-5′,7′
128.29	CH-11′,13′
128.87	CH-4′,8′
129.02	CH-10′,14′
129.49	CH-6′
130.31	C_q-6
130.76	CH-12′
132.72	C_q-3′
134.82	C_q-9′
140.94	CH-5
146.15	CH-3
148.24,148.67	C_q-8, 9
164.60	C_q-2′
164.68	C=O-1

2.2.5.2 Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid diphenylmethyl amide (84)



To a solution of piperic acid (0.5001 g, 2.3 mmol) in dried THF (4 ml) at room temperature under nitrogen atmosphere was added oxalyl chloride (1 ml, 11.5 mmol) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride as an orange residue. To the crude piperic acid chloride in dried THF (4 ml), was added the solution of diphenylmethanamine (0.4192 g, 2.3 mmol) in dried THF (3 ml) followed by triethylamine (0.48 ml, 3.4 mmol) and stirred at 70 ºC for 5 h. The reaction mixture was evaporated to give the yellow crude product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:1, as eluent) to obtain compound **84** (0.3809 g, 43.35 % yield) as light yellow crystals from CH_2Cl_2/hexane, m.p. 156.5-157.1 ºC, 83 % conversion from the starting material.

The structure of compound **84** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 13.



84

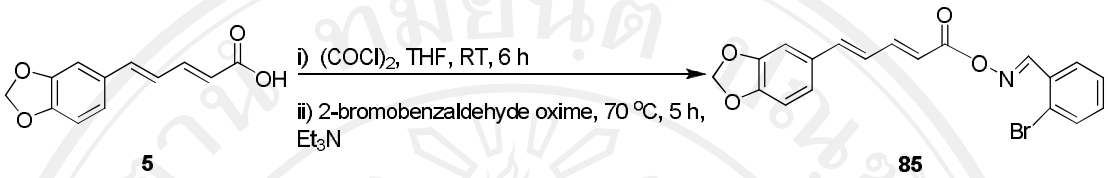
Table 13 Data of compound **84**

Physical properties	
Light yellow crystals m.p. 156.5-157.1 ºC (CH_2Cl_2/hexane)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3283	N-H stretch of secondary amine group
2936	C-H stretch of benzene ring
2950, 2830	C-H stretch of CH_2
1675	C=O stretch of amide group
1600, 1490	C=C stretch of the benzene ring
1396	C-N stretch
1034, 1255	C-O stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{25}H_{21}NO_3$	383.1521 $(M)^+$
Calc. mass for $C_{25}H_{21}NO_3Na$	406.1419 $(M+Na)^+$
Found for $C_{25}H_{22}NO_3$	384.1599 $(M+H)^+$

Table 13 (continued)

NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
5.32	1H, *s,* NH
5.99	1H, *d* (*J* = 2.00 Hz), H-2
6.00	2H, *s*, H-12
6.02	1H, *s*, H-2′
6.65-6.73	1H, *m*, H-4
6.79-6.86	2H, *m*, H-5,10
7.00	1H, *d* (*J* = 1.20 Hz), H-7
6.37-6.39, 7.26-7.31, 7.34-7.40	10H, *m*, H-4′,5′,6′,7′,8′,10′,11′,12′,13′,14′
7.42	1H, *dd* (*J* = 14.81, 10.81 Hz), H-3
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
56.60	C_q-2′
100.86	CH_2-12
106.32	CH-7
108.19	CH-10
122.02	CH-2
122.20	CH-11
123.99	CH-4
126.98	CH-5′,6′,7′,11′,12′,13′
128.18	CH-4′,8′,10′,14′
130.28	C_q-6
138.85	CH-5
141.00	CH-3
144.45	C_q-3′,9′
147.72,147.98	C_q-8,9
164.66	C=O-1

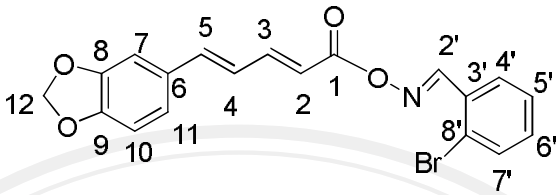
2.2.5.3 Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid 2-bromobenzaldehyde oxime ester (85)



5 85

To a solution of piperic acid (0.5002 g, 2.3 mmol) in dried THF (4 ml) at room temperature under nitrogen atmosphere was added oxalyl chloride (1 ml, 11.5 mmol) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride as an orange residue. To the crude piperic acid chloride in dried THF (4 ml), was added the solution of 2-bromobenzaldehyde oxime (0.4587 g, 2.3 mmol) in dried THF (3 ml) followed by triethylamine (0.48 ml, 3.4 mmol) and stirred at 70 ºC for 5 h. The reaction mixture was evaporated to give the yellow crude product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 1:9, as eluent) to obtain compound **85** (0.2684 g, 29.27 % yield) as yellow crystals from CH_2Cl_2/hexane, m.p. 175.8-176.4 ºC, 76 % conversion from the starting material.

The structure of compound **85** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 14.



85

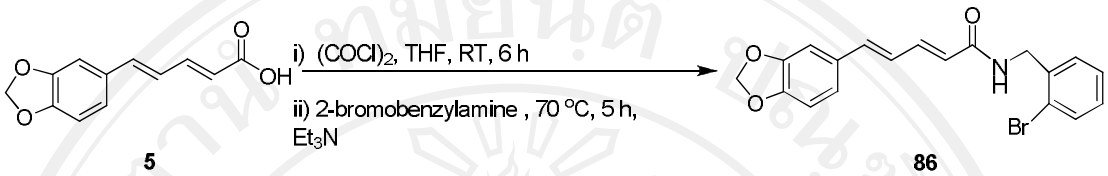
Table 14 Data of compound **85**

Physical properties	
Yellow crystals m.p. 175.8-176.4 ºC (CH_2Cl_2/hexane)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3050, 3100	C-H stretch of benzene ring
2950, 2830	C-H stretch of CH_2
1690	C=O stretch
1630	C=C stretch of the conjugated double bonds
1600, 1500	C=C stretch of the benzene ring
1050, 1250	C-O stretch
698,738	C-Br stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{19}H_{14}BrNO_4$	399.0106 $(M)^+$
Calc. mass for $C_{19}H_{14}NO_4$	321.1001 $(M\text{-}Br)^+$
Found for $C_{19}H_{14}NO_4$	321.1004 $(M\text{-}Br)^+$

Table 14 (continued)

NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
6.00	2H, *s*, H-12
6.08	1H, *d* (*J* = 15.21 Hz), H-2
6.76-6.88	3H, *m*, H-4,5,10
6.90-6.98	1H, *m*, H-11
7.03	1H, *d* (*J* = 1.60 Hz), H-7
7.32-7.37	3H, *m*, H-5′,6′,7′
7.61-7.63	1H, *m*, H-3
8.09-8.15	1H, m, H-4′
8.84	1H, *s*, H-2′
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
101.2	CH_2-12
106.7	CH-7
108.4	CH-10
117.2	CH-2
121.6	CH-8′
122.5	CH-11
125.2	CH-4
127.1	CH-4′
127.8	CH-5′
130.1	CH-6′
130.5	C_q-6
132.8	CH-7′
135.3	C_q-3′
138.4	CH-5
147.2	CH-3
148.0, 148.7	C_q-8, 9
153.8	CH-2′
171.5	C=O-1

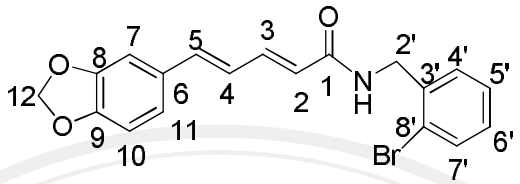
2.2.5.4 Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid 2-bromobenzyl amide (86)



5 86

To a solution of piperic acid (0.4999 g, 2.3 mmol) in dried THF (4 ml) at room temperature under nitrogen atmosphere was added oxalyl chloride (1 ml, 11.5 mmol) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride as an orange residue. To the crude piperic acid chloride in dried THF (4 ml), was added the solution of 2-bromobenzylamine (0.4263 g, 2.3 mmol) in dried THF (3 ml) followed by triethylamine (0.48 ml, 3.4 mol) and stirred at 70 ºC for 5 h. The reaction mixture was evaporated to give the yellow crude product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:1, as eluent) to obtain compound **86** (0.3657 g, 41.32 % yield) as yellow crystals from CH_2Cl_2/hexane, m.p. 261.3-263.1 ºC, 81 % conversion from the starting material.

The structure of compound **86** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 15.



86

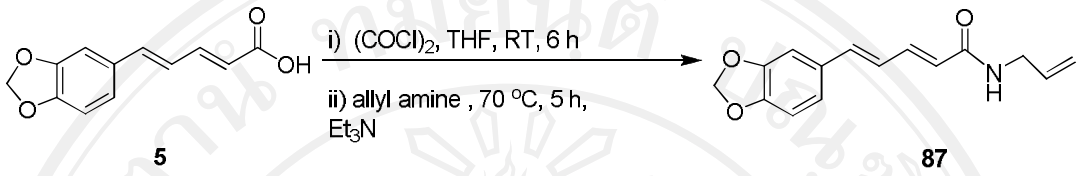
Table 15 Data of compound **86**

Physical properties	
Yellow crystals m.p. 261.3-263.1 ºC (CH_2Cl_2/hexane)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3263 3050, 3100 1655 1600, 1500 1050, 1250	N-H stretch of secondary amine group C-H stretch of benzene ring C=O stretch of amide group C=C stretch of the benzene ring C-O stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{19}H_{16}BrNO_3$ Calc. mass for $C_{19}H_{16}BrNO_3Na$ Found for $C_{19}H_{17}BrNO_3$	385.0314 $(M)^+$ 408.0211 $(M+Na)^+$ 386.0392 $(M+H)^+$

Table 15 (continued)

NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
3.74	1H, *br*, NH
4.53	2H, *d* (*J* = 5.60 Hz), H-2′
5.93	1H, *d* (*J* = 14.81 Hz), H-2
5.97	2H, *s*, H-12
6.63-6.67	1H, *m*, H-4
6.76-6.79	2H, *m*, H-5,10
6.88	1H, *d* (*J* = 8.40 Hz), H-11
6.97	1H, *s*, H-7
7.27-7.34	3H, *m*, H-4′,5′,6′
7.35-7.40	1H, *m*, H-3
7.78	1H, *d* (*J* = 6.09 Hz), H-7′
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
43.78	CH_2-2′
101.32	CH_2-12
105.75	CH-7
108.50	CH-10
122.67	CH-2
122.78	C_q-8′
124.61	CH-11
127.53	CH-4
127.91	CH-5′
128.73	CH-6′
130.07	CH-4′
130.83	C_q-6
132.43	CH-7′
138.34	CH-5
139.13	CH-3
141.52	C_q-3′
148.23, 148.29	C_q-8, 9
166.06	C=O-1

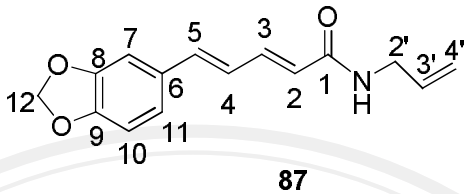
2.2.5.5 Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid allyl amide (87)



5 87

To a solution of piperic acid (0.5002 g, 2.3 mmol) in dried THF (4 ml) at room temperature under nitrogen atmosphere was added oxalyl chloride (1 ml, 11.5 mmol) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride as an orange residue. To the crude piperic acid chloride in dried THF (4 ml), was added the solution of allyl amine (0.1308 g, 2.3 mmol) in dried THF (3 ml) followed by triethylamine (0.48 ml, 3.4 mmol) and stirred at 70 ºC for 5 h. The reaction mixture was evaporated to give the yellow crude product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:1, as eluent) to obtain compound **87** (0.3473 g, 58.91 % yield) as slightly yellow crystals from CH_2Cl_2/hexane, m.p. 181.8-182.5 ºC, 86 % conversion from the starting material.

The structure of compound **87** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 16.



87

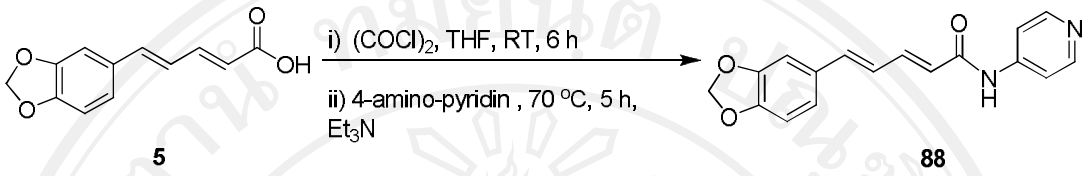
Table 16 Data of compound **87**

Physical properties	
Slightly yellow crystals m.p. 181.8-182.5 ºC (CH_2Cl_2/hexane)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3259	N-H stretch of secondary amine group
3067, 2907	C-H stretch of benzene ring
2950, 2830	C-H stretch of CH_2
1645	C=O stretch of amide group
1038, 1253	C-O stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{15}H_{15}NO_3$	257.1130 $(M)^+$
Calc. mass for $C_{15}H_{15}NO_3Na$	280.0950 $(M+Na)^+$
Found for $C_{15}H_{15}NO_3Na$	280.0956 $(M+Na)^+$

Table 16 (continued)

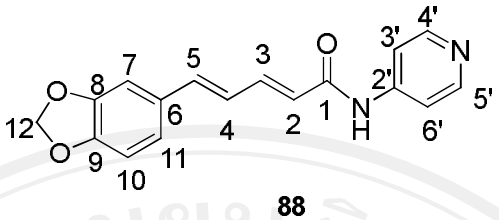
NMR spectroscopy	
^{1}H NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of protons
3.98	2H, *t* (*J* = 5.76, 1.38 Hz), H-2′
5.14	1H, *dd* (*J* = 8.87, 1.25 Hz), H-4′
5.21	1H, *dd* (*J* = 17.15, 1.44 Hz), H-4′
5.82-5.92	2H, *m*, NH, H-3′
5.95	3H, *s*, *d* (*J* = 10.00 Hz), H-12,2
6.63-6.71	1H, *m*, H-4
6.74-6.77	2H, *m*, H-5,10
6.86	1H, *dd* (*J* = 8.05, 1.49 Hz), H-11
6.95	1H, *d* (*J* = 1.49 Hz), H-7
7.36	1H, *dd* (*J* = 15.00, 10.00 Hz), H-3
^{13}C NMR (400 MHz) in $CDCl_3$	
Chemical shift (δ, ppm)	Type of carbons
42.03	CH_2-2′
101.26	CH_2-12
105.69	CH-7
108.44	CH-10
116.41	CH-4′
122.57	CH-2
122.87	CH-11
124.58	CH-4
130.77	C_q-6
134.21	CH-3′
138.97	CH-5
141.25	CH-3
148.15, 148.20	C_q-8, 9
166.01	C=O-1

2.2.5.6 Preparation of 5-(3,4-methylenedioxyphenyl)-2E,4E-pentadienoic acid 4-amino-pyridin amide (88)



To a solution of piperic acid (0.5000 g, 2.3 mmol) in dried THF (4 ml) at room temperature under nitrogen atmosphere was added oxalyl chloride (1 ml, 11.5 mmol) and stirred at room temperature for 6 h. The reaction mixture was evaporated to give the crude piperic acid chloride as an orange residue. To the crude piperic acid chloride in dried THF (4 ml), was added the solution of 4-amino-pyridin (0.1850 g, 2.3 mmol) in dried THF (3 ml) followed by triethylamine (0.48 ml, 3.4 mmol) and stirred at 70 ºC for 5 h. The reaction mixture was evaporated to give the yellow crude product and then purified by preparative thin layer column chromatography (silica gel; ethyl acetate in hexane, 2:1, as eluent) to obtain compound **88** (0.4803 g, 53.16 % yield) as yellow crystals from CH_2Cl_2/hexane, m.p. 171.5-172.4 ºC, 84 % conversion from the starting material.

The structure of compound **88** was characterized by ^{1}H NMR, ^{13}C NMR, IR and MS and the reaction conditions for the preparation and the spectroscopic data were shown Tables 17.



88

Table 17 Data of compound **88**

Physical properties	
Yellow crystals m.p. 171.5-172.4 ºC (CH_2Cl_2/hexane)	
IR Spectroscopy (Evaporated thin film)	
Frequency (υ,cm^{-1})	Type of vibrations
3440	N-H stretch of secondary amine group
1692	C=O stretch of amide group
1599, 1509	C=C stretch of the benzene ring
1372	C-N stretch
1039, 1255	C-O stretch
Mass spectrometry (ESI-MS)	
Molecular weight	m/z
Calc. mass for $C_{16}H_{19}NO_3$	294.1004 $(M)^+$
Calc. mass for $C_{16}H_{19}NO_3Na$	317.0902 $(M+Na)^+$
Found for $C_{16}H_{19}NO_3Na$	317.0907 $(M+Na)^+$

Table 17 (continued)

NMR spectroscopy	
^{1}H NMR (400 MHz) in DMSO-d6	
Chemical shift (δ, ppm)	Type of protons
6.06	1H, *s*, H-12
6.28	1H, *d* (*J* = 14.92 Hz), H-2
6.90-6.95	1H, *m*, H-4
6.97-7.16	3H, *m*, H-5,10,11
7.31	1H, *d* (*J* = 1.47 Hz), H-7
7.35-7.41	1H, *m*, H-3
7.61-7.69	2H, *m*, H-3′,6′
8.42	2H, *d* (*J* = 5.27 Hz), H-4′,5′
10.48	1H, *s,* NH
^{13}C NMR (400 MHz) in DMSo-d6	
Chemical shift (δ, ppm)	Type of carbons
101.38	CH_2-12
105.81	CH-7
108.51	CH-6′
108.96	CH-3′
113.24	CH-10
123.22	CH-2
123.32	CH-11
124.90	CH-4
130.64	C_q-6
139.98	CH-5
142.51	CH-3
145.93	C_q-2′
148.02, 148.11	C_q-8, 9
150.34	CH-4′,5′
164.88	C=O-1

2.2.6 Bioactivities testing of the piperine derivatives

2.2.6.1 Antibacterial activity[33,39]

The antibacterial activities of the extracts were determined using the paper disc method. The bacteria used were: *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923 (Gram positive bacteria), *Pseudomonas aeruginosa* ATCC27553 (Gram negative bacteria) and *Salmonella typhimurium* ATCC13311.

In brief, a loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 h to activate the strain. Mueller Hinton Agar was prepared for the study. The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (0.2 ml) was inoculated into the media (inoculums size 108 cells/ml) when the temperature reached 40-42 ºC. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions.

The compounds **1, 5, 83, 84, 85, 86, 87** and **88** were weighed and dissolved in dimethylsulfoxide (DMSO) to make a solution of concentration 10, 50, 100 mg/ml. Sterilized filter discs were dipped in these solutions and subsequently dried to remove DMSO. Mueller–Hinton agar was prepared and allowed to solidify. One of these discs was kept free from antibiotic and served as growth control. Five different bacteria were selected and 1 ml of each bacterial culture broth were added in the Mueller–Hinton plates and spread with the help of sterile spreader. The filter paper discs soaked in above-mentioned dilutions of compounds number **1, 5, 83, 84, 85, 86, 87** and **88** were placed aseptically over the inoculated plates using sterile forceps. 0.75 mg/ml of Gentamicin and 20 μL of 100% DMSO were used as a positive control.

The plates were incubated at 37 ºC for 24 h, in upright position. The zone of inhibition was measured.

The result of antibacterial activity of the compounds **1, 5, 83, 84, 85, 86, 87** and **88** were shown in Table 18.

Table 18 The antibacterial activity of the compounds **1, 5, 83, 84, 85, 86, 87** and **88**

Compound	Conc. (ppm)	Size of zone inhibition (cm)			
		E.coli	***S.aureus***	***P.aeroginosa***	***S.typhimurium***
1	10	-	-	-	-
	50	-	-	-	-
	100	-	-	0.7	-
5	10	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
83	10	-	-	-	-
	50	-	-	-	-
	100	0.8	-	-	-
84	10	-	-	0.8	0.8
	50	-	-	0.9	0.8
	100	0.9	-	1.0	0.8
85	10	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
86	10	-	-	-	0.9
	50	1.0	-	0.7	1.0
	100	1.0	-	0.8	1.1

Table 18 (continued)

Compound	Conc. (ppm)	Size of zone inhibition (cm)			
		E.coli	***S.aureus***	***P.aeroginosa***	***S.typhimurium***
87	10	0.8	-	-	-
	50	1.0	-	0.6	-
	100	1.0	-	0.7	-
88	10	0.6	-	0.6	-
	50	0.6	-	0.6	-
	100	1.0	-	0.6	0.8
DMSO	-	-	-	-	-
Gentamycin	-	2.3	3.2	1.8	2.4

Note

- Inactive

2.2.6.2 Antifungal activity[32]

Antifungal activity was measured by paper disc method. The fungal used were *Candida albicans* and *Candida krusei* (yeast).

A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 h to activate the strain. The each fungal were incubated in yeast-maltose agar broth for 20 hours at 37 °C at the standing condition. The media and the test fungal cultures were poured into Petri dishes (Hi-Media). The test strain (0.2 ml) was inoculated into the media (inoculums size 108 cells/ml) when the temperature reached 40-42 ºC. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions.

The compounds **1, 5, 83, 84, 85, 86, 87** and **88** were weighed and dissolved in dimethylsulfoxide (DMSO) to make a solution of concentration 10, 50, 100 mg/ml. Sterilized filter discs were dipped in these solutions and subsequently dried to remove DMSO. Yeast-maltose agar was prepared and allowed to solidify. One of these discs was kept free from antibiotic and served as growth control. Two different funguses were selected and 1 ml of each funguses culture broth were added in a plates and spread with the help of sterile spreader. The filter paper discs soaked in above-mentioned dilutions of compounds number **1, 5, 83, 84, 85, 86, 87** and **88** were placed aseptically over the inoculated plates using sterile forceps. 0.75 mg/ml of Gentamicin and 20 μL of 100% DMSO were used as a positive control. The plates were incubated at 37 ºC for 24 h, in upright position. The zone of inhibition was measured.

The result of antifungal of the compounds **1, 5, 83, 84, 85, 86, 87** and **88** were shown in Table 19.

Table 19 The antifungal activity of the compounds **1, 5, 83, 84, 85, 86, 87** and **88**

Compound	Conc. (mg/ml)	Size of zone inhibition (cm)	
		C.albicans	***C.krusei***
1	10	-	-
	50	-	-
	100	-	-
5	10	-	-
	50	-	-
	100	-	-
83	10	-	-
	50	-	-
	100	-	-
84	10	-	-
	50	1.0	-
	100	1.1	-
85	10	-	-
	50	-	-
	100	-	-
86	10	1.0	-
	50	1.0	-
	100	1.0	-
87	10	-	-
	50	0.7	-
	100	0.8	-
88	10	-	-
	50	1.2	-
	100	1.3	-
DMSO	-	-	-
Gentamycin	-	-	-

Note

- Inactive

2.2.6.3 Antioxidant activity[40]

The compounds **1, 5, 83, 84, 85, 86, 87** and **88** were subjected to thin layer chromatography study. The plates were sprayed by 0.2 mM DPPH in methanol solution for 5 seconds and images were observed under visible light at exactly 2 min after spraying. The area of bright yellow bands against the purple background then determined radical scavenging activity

The result of antioxidant of the compounds **1, 5, 83, 84, 85, 86, 87** and **88** were shown in Table 20.

Table 20 DPPH radical scavenging activities of compounds **1, 5, 83, 84, 85, 86, 87** and **88**

Compounds	Color bands	Activity
1	purple	Inactive
5	purple	Inactive
83	purple	Inactive
84	purple	Inactive
85	purple	Inactive
86	purple	Inactive
87	purple	Inactive
88	purple	Inactive