SYNTHESIS AND BIOACTIVITY STUDIES OF COUMARIN AND ITS DERIVATIVES

WONG KOK TONG

UNIVERSITI TEKNOLOGI MALAYSIA

SYNTHESIS AND BIOACTIVITY STUDIES OF COUMARIN AND ITS DERIVATIVES

WONG KOK TONG

A thesis submitted in fulfilment of the
Requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

JULY 2013

Dedicated to my beloved family

ACKNOWLEDGEMENT

First and foremost, praise to the Buddha, Dhamma and Sangka.

I would like to extent my sincerest appreciation to my supervisor, Dr. Norazah binti Basar and Dr. Razauden bin Mohamed Zulkifli for their guidance, help, inspiration, attention, encouragement and advices to accomplish throughout my work. Not forgotten to Assoc. Prof. Dr. Farediah binti Ahmad for her invaluable assistance and continuous supports throughout this project.

My appreciation also goes to all the academic staff of Chemistry Department, all laboratory staff (Mdm. Ain, Mr. Azmi, Mr. Rasyidi and Mdm. Nasrena), and fellow friends (Athirah, Amira, Atikah, Atiqah, Awanis, Kiew Siaw Fui, Mariam, Noraimi and others) willing to lend a helping hand.

It is a pleasure to pay tribute also to Dr. Lisa Harris of University College London for the Mass Spectrum Analysis. I would also acknowledge Ministry of Higher Education Malaysia (MOHE) for giving me the scholarship to pursue my Master degree at Universiti Teknologi Malaysia.

Furthermore, my utmost grateful to my parents (Wong Soon See and Wong Yoke Hun) and my entire family (Wong Kok Wei, Chow Chui See, Wong Kok Yeong and Wong Pei Yee) for their encouragement and valuable supports. Last but not least, I would also to thank who are involved directly and indirectly in the completion of this study.

PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between July 2010 and August 2012 under the supervision of Dr. Norazah Basar and Dr Razauden bin Mohamed Zulkifli. Parts of my work described in this thesis have been reported in the following publications:

1. Kok Tong Wong and Norazah Basar (2012). Coumarins *via* Knoevenagel Condensation Reaction (KCR) and Pechmann Condensation Reaction. National Science Postgraduate Conference 2011 (NSPC), 16-17 November 2011, at Ibnu Sina Institute, UTM, Johor Bahru, Malaysia. *Jurnal Teknologi*. **57**: 83-98.

2. Kok Tong Wong, Norazah Basar and Razauden Mohamed Zulkifli (2012). Synthesis of Substituted Coumarin. Paper presented at the 2nd National Symposium In Organic Synthesis 2012 (NaSOS II) at Concorde Hotel, Shah Alam, Selangor. 16-17 July 2012.

ABSTRACT

Coumarin is a naturally occurring compounds being present in several plants and also can be produced through organic synthetic reactions. In this study, substituted coumarins of 3-acetylcoumarin, 3-acetyl-7-(diethylamino)coumarin, 7-(diethylamino)-3-(1-oxobutyl)coumarin, 3-oxobutyl-*3H*-naphtho[2,1-b]pyran-2-one, 6-bromo-3-(1-oxobutyl)coumarin and 8-methoxy-3-(1-oxobutyl)coumarin were synthesized *via* Knoevenagel condensation reaction of respective 2-hydroxy-benzaldehyde derivatives with active methylene group from ethyl acetate or ethyl butyrylacetate under basic condition. Meanwhile, 7-hydroxy-4-methylcoumarin, 4-methyl-2*H*-benzo[*h*]chromen-2-one, 7-hydroxy-4,8-dimethylcoumarin, 7-hydroxy-4-propylcoumarin, 4-propyl-2*H*-benzo[*h*]chromen-2-one, 7-hydroxy-8-methyl-4-propylcoumarin and 7,8-dihydroxy-4-propylcoumarin were prepared through Pechmann condensation reaction by the condensation of respective substituted phenol and β-keto-ester in the presence of sulphuric acid as a catalyst. Futher methylation reaction on 7-hydroxy-4-methylcoumarin with iodomethane catalysed by K_2CO_3 gave 7-methoxy-4-methylcoumarin. Modification of hydroxyl group of 7,8-dihydroxy-4-propylcoumarin using butyric anhydride and dry pyridine yielded 7,8-bis-(1-oxobutoxy)-4-propylcoumarin. In addition, 7-hydroxy-4,8-dimethyl-coumarin was converted to 7-benzyloxy-4,8-dimethylcoumarin through reaction of benzyl chloride in dry acetone. In addition, 7,8-methylenedioxy-4-propylcoumarin undergoes Williamson etherification reaction which involved S_N2 mechanism between secondary alkyl halide with potassium salt of a phenoxide. All compounds were characterized by spectroscopic techniques using infrared (IR), proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR), and mass spectrometry (MS). The synthesized compounds were tested for their antioxidant and antibacterial activities. It was found that the hydroxylated coumarin derivatives of 7,8-dihydroxy-4-propylcoumarin were tested positive towards 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay with IC_{50} value of 4.09 μg/mL while other hydroxylated compounds were inactive. The antibacterial activity show that 6-bromo-3-(1-oxobutyl)coumarin exhibited as strong antibacterial agent against *B. subtilis* bacteria with MIC value 56.25 μg/mL. 7,8-Methylenedioxy-4-propylcoumarin showed strong activity to all Gram-positive bacteria.

ABSTRAK

Kumarin adalah sebatian semulajadi yang terdapat dalam tumbuhan dan juga boleh dihasilkan melalui tindak balas sintesis organik. Dalam kajian ini, kumarin tertukarganti iaitu 3-asetilkumarin**,** 3-asetil-7-(dietilamino)kumarin, 7-(dietilamino)-3-(1-oksobutil)kumarin, 3-oksobutil-*3H*-naftol[2,1-b]piran-2-on, 6-bromo-3-(1-oksobutil)kumarin dan 8-metoksil-3-(1-oksobutil)kumarin disintesis melalui tindak balas kondensasi Knoevenagel (KCR) menggunakan terbitan 2-hidroksibenzaldehid dengan kumpulan metilena yang aktif daripada etil asetoasetat atau etil butirilasetat dalam keadaan bes. Sementara itu, 7-hidroksi-4-metilkumarin, 4-metilbenzo[*h*]kumarin, 7-hidroksi-4,8- dimetilkumarin, 7-hidroksi-4-propilkumarin, 4-propilbenzo[*h*]kumarin, 7-hidrosil-8-metil-4-propilkumarin dan 7,8-dihidroksil-4-propilkumarin disediakan melalui tindak balas kondensasi Pechmann ke atas fenol tertukarganti dan β-keto ester dengan kehadiran asid sulfurik sebagai mangkin. Tindak balas pemetilan ke atas 7-hidroksi-4-metilkumarin dengan metil iodida dimangkinkan oleh K_2CO_3 memberikan 7-metoksi-4-metilkumarin. Pengubahsuaian ke atas kumpulan hidroksil bagi 7,8-dihidroksi-4-metilkumarin menggunakan butirik anhidrida dan piridina kering menghasilkan 7,8-bis-(1-oksobutoksi)-4-propilkumarin. 7-Hidroksi-4,8- dimetilkumarin telah diubah kepada 7-benziloksi-4,8-dimetilkumarin melalui tindak balas dengan benzil klorida dalam pelarut aseton kering. Di samping itu, 7,8-metilenedioksi-4-propilkumarin disediakan melalui tindak balas eterifikasi Williamson melalui mekanisme S_N2 di antara alkil halida sekunder dan garam kalium fenoksida. Semua hasil tindak balas dicirikan dengan kaedah spektroskopi inframerah (IM), resonans magnet nukleus (RMN ^{1}H and ^{13}C) dan spektrometri jisim (SJ). Sebatian yang disintesis telah melalui ujian aktiviti antioksida dan antibakteria. Terbitan hidroksi kumarin iaitu 7,8-dihidroksi-4-propilkumarin menunjukkan aktiviti positif terhadap 2,2-difenil-1-pikrilhidrazil (DPPH) dengan nilai IC_{50} 4.09 μg/mL manakala sebatian hidroksi yang lain didapati tidak aktif. Hasil ujian aktiviti antibakteria menunjukkan 6-bromo-3-(1-oksobutil)kumarin dikenalpasti sebagai agen antibakteria yang kuat terhadap bakteria *B. subtilis* dengan nilai MIC sebanyak 56.25 μg/mL. Manakala 7,8-metilenedioksi-4-propilkumarin menunjukkan aktiviti antibakteria yang kuat kepada semua bakteria Gram-positif dalam cerakinan antibakteria.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	arachidonic acid
AIDS	acquired immune deficiency syndrome
ATR	attenuated total reflectance
$BaCl_2$	barium (II) chloride
^{13}C	carbon-13
$CaCO_3$	calcium carbonate
$CaCl_2$	calcium chloride
$CDCl_3$	deuterated chloroform
CD_3COCD_3	deuterated acetone
CD_3OD	deuterated methanol
CH_2I_2	diiodomethane
CH_3I	iodomethane
CIMS	Chemical Impact Mass Spectrometry
d	doublet
dd	doublet of doublet
DDM	disc diffusion method
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EIMS	Electron Impact Mass Spectrometry
FRAP	Ferric Reducing/ Antioxidant Power

hr	hour
^{1}H	proton
HIV	human immunodeficiency virus
HNO_3	nitric acid
H_2SO_4	sulphuric acid
Hz	hertz
I%	percentage of inhibition
IC_{50}	inhibition concentration at 50%
IR	infrared
$InCl_3$	indium (III) chloride
J	coupling constant
KBr	potassium bromide
KCR	Knoevenagel condensation reaction
K_2CO_3	potassium carbonate
lit	literature
m/z	mass to charge ratio
m	multiplet
m.p	melting point
m-ZrP	mesoporous zirconium phosphate
MBC	minimum bactericidal concentration
MIC	minimum inhibition concentration
MS	mass spectrometry
n-BuOAc	n-butyl acetate
NA	nutrient agar
NB	nutrient broth
NMR	nuclear magnetic resonance
ORAC	Oxygen Radical Absorbance Capacity
PAF	platelet activating factor
PSC	Peroxyl Radical Scavenging Capacity

PVPP-BF_3	poplyvinylpolypyrrolidone-bound boron trifluoride
q	quartet
s	singlet
S_N2	Second-Order Nucleophilic Substitution reaction
SD	standard deviation
SS	streptomycin sulphate
r.t	room temperature
R_f	retention factor
t	triplet
T3P	propylphosphonic anhydride
TBATB	*n*-tetrabutylammonium tribromide
TEAC	Trolox Equivalent Antioxidant Capaciy
TGMT	1,1,3,3,-*N,N,N',N'*-tetramethylguanidinium trifluoroacetate ionic liquid
THF	tetrahydrofuran
TLC	thin layer chromatography
[TMPSA][HSO_4]	*N*,*N*,*N*-Trimethyl-*N*-propanesulfonic acid ammonium hydrogen sulfate
TOSC	Total Oxyradical Scavenging Capacity
TRAP	Total Radical-Trapping Antioxidant Parameter
TSIL	task-specific ionic liquids
UV-Vis	ultraviolet visible
$ZrOCl_3$	zirconium salt
Zr-TMS	zirconia based transition metal oxide mesoporous molecular sieve
α	alpha
β	beta
δ	chemical shift

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

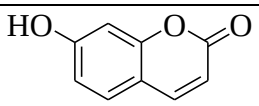
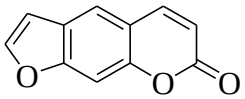
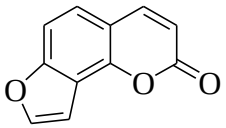
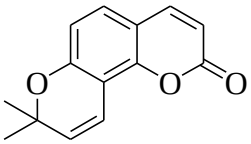
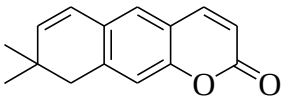
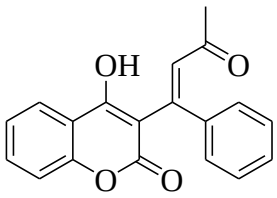
1.1 Introduction

Coumarins (benzopyrones) are simple molecules that contain two structures of six member heterocyclic rings with two oxygen atoms. The coumarins can be classified as simple coumarin, furanocoumarins, pyranocoumarins and coumarins substituted in the pyrone ring (**Table 1.1**) [1]. Simple coumarins can undergoes hydroxylation, alkoxylation and alkylation to form its derivatives. For furanocoumarins, these compounds consist of five-member furan ring attached to the coumarin structure. Pyranocoumarins is a compound that contained a linear or angular type with substituents on benzene and pyrone rings.

Coumarins are naturally occurring important plant constituents and occupy unique in natural and organic synthesis chemistry [2]. Coumarin can be found in several plants especially in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), woodruff (*Galium odoratum*), mullein (*Verbascum* spp.), and sweet grass (*Hierochloe odorata*). Coumarin has a sweet scent and had been recognised as the scent of newly-mown hay. Because of the sweet scent, coumarin has been used in perfumes since 1882 [3]. Coumarins also can be produced through organic synthetic reactions although it can be found naturally in several green plants. Coumarins and its derivatives can be synthesized through Knoevenagel condensation reaction (KCR), Pechmann condensation reaction, Perkin

reaction, Wittig reaction, Claisen, Reformatsky and Kontanecki-Robinson reaction [4].

Table 1.1: The main structural features and examples of four subtype of coumarin.

Classification	**Features**	**Examples**
Simple coumarins	Hydroxylated, alkoxylated or alkylated on benzene ring	 7-Hydroxycoumarin
Furanocoumarins	5-membered furan ring attached to benzene ring Linear or angular	 Psoralen  Angelicin
Pyranocoumarins	6-membered pyran ring attached to benzene ring Linear or angular	 Seselin  Xanthyletin
Pyrone-substituted coumarins	Substitution on pyrone ring, often at 3-C or 4-C positions	 Warfarin

Nowadays, coumarins play an important role as food constituents [5], and dye-sensitized solar cells [6]. Coumarins also used as additives to cigarettes [7]. In addition, coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances [8]. Coumarin and its derivatives were successfully made great interesting targets for organic chemist due to their chemical properties. The pharmacological and biochemical properties and also the applications of therapeutics will be affected due

to the different position of coumarin substitution. Thus, it is utmost important to synthesis coumarins and their derivatives through a simple and effective method.

1.2 Problem Statements

Coumarins can be isolated from plants naturally but with the low percentage of yields. Thus, this problem limits the use of coumarins in various applications especially for bioactivity purposes. Many synthesis methods have been developed to synthesize the coumarins and its derivatives such as Knoevenagel and Pechmann condensation reactions. Both of these methods were used in this study which offered convenience synthetic procedures to access coumarin derivatives which are not found naturally. The synthetic compounds may provide interesting biological properties especially in antioxidant and antibacterial studies.

1.3 Research Objectives

The objectives of this study are:

1. To synthesize and characterize coumarin derivatives

2. To carry out derivatization reaction of the synthesized coumarin.

3. To screen the antioxidant and antibacterial activities of coumarin derivatives.

1.4 Scope of Study

In this study, two methods were used to synthesize coumarin derivatives which are Knoevenagel condensation reaction (KCR) and Pechmann condensation reaction. Coumarin derivatives were synthesized by KCR which involved the condensation of an aldehyde with a compound containing an active methylene group in the presence of catalytic amount of base. Coumarin derivatives also can be synthesized through Pechmann condensation reaction which involved the condensation of substituted phenols with β-ketoester in the presence of an acid catalyst. The synthesized coumarins were undergoes derivatization reaction to synthesize several type of coumarin derivatives such as methylation, esterification, benzylation and ketalation. All compounds were characterized by spectroscopic techniques using infrared (IR), proton and carbon-13 nuclear magnetic resonance (^{1}H and ^{13}C NMR), and mass spectrometry (MS). Screening of antioxidant and antibacterial activities for the synthesized coumarin derivatives were carried out.

1.5 Significant of Study

Coumarins have strong biochemical and pharmacological properties. Coumarins and its derivatives are very useful in medicine field such as for the treatment of lymphoedema, and in treatment of cancer (breast, lung and kidney cancer) [9]. This research is vital to synthesize the coumarin compounds by using KCR and Pechmann reaction. These will provide the alternative way to afford coumarin and its derivatives as compared to natural resources. Screening of biological activities such as antioxidant and antibacterial activities for the synthesized coumarin derivatives were carried out indicated the useful of these compounds.

REFERENCES

[1] Lacy, A. and Kennedy, R. O'. (2004). Studies On Coumarin-Related Compounds To Determine Their Therapeutic Role In The Treatment Of Cancer. *Current Pharmaceutical Design*, **10**: 3797-3811.

[2] Sunil, K. B., Kumar, P. S., Srinivasulu, N., Rajitha, B., Thirupathi Reddy, Y., Narsimha Reddy, P. and Udupi, R. H. (2006). Vanadium(III) Chloride As An Effective Catalyst For The Pechmann Reaction. *Chemistry of Heterocyclic Compounds*, **42**(2)**:** 172-175.

[3] Aslam, K., Khosa, M. K., Jahan, N. and Nosheen, S. (2010). Synthesis and Applications of Coumarin. Pakistan: *Journal of Pharmaceutical Science*. **23** (4): 449-454.

[4] Ojala, T. (2001). *Biological Screening of Plants Coumarins*. Academic Dissertation. Faculty of Science of the University of Helsinki.

[5] Kennedy, R. O'. and Thornes, R. D. (1997). *Coumarin: Biology, Application And Modes Of Action.* New York: Wiley & Sons. 1-64.

[6] Hara, K., Sayama, K., Ohga, Y., Shinpo, A., Suga, S. and Arakawa, H. (2001). A Coumarin Derivative Dye Sensitized Nanocrystalline TiO_2 Solar Cell Having A High Solar Energy Conversion Efficiency Up To 5.6%. *Chemical Communications*. 569-570.

[7] Givel, M. (2003). *A Comparison Of US And Norwegian Regulation Of Coumarin In Tobacco Products: Tobacco Control.* Oklahnoma, BMJ Publishing Group Ltd. **12**: 401-405.

[8] Kostova, I. (2005). Synthetic and Natural Coumarins as Cytotoxic Agents. *Current Medicinal Chemistry - Anti-Cancer Agents.* Bulgaria: Bentham Science Publishers Ltd. **5**: 29-46.

[9] Anonymous (2000). Coumarin: *IARC Monographs Evaluation Carcinogenic Risk Chemicals to Human*. **77**: 193-225

[10] Al-Bayati, R. I., Al-Amiery, A. A. H. and Al-Majedy, Y. K. (2010). Design, Synthesis and Bioassay of Novel Coumarins. *African Journal of Pure and Applied Chemistry*, **4**(6): 74-86.

[11] Jain, P. K. and Joshi, H. (2012). Coumarin: Chemical and Pharmacological Profile. *Journal of Applied Pharmaceutical Science*. **2**(6): 236-240).

[12] Lake, B. G. (1999). Coumarin Metabolism, Toxicity and Carcinogenicity: Relevance for Human Risk Assessment. *Food and Chemical Toxicology*. **37**: 423-453.

[13] Chaturvedi, R. and Mulchandani, N. B. (1989). Coumarins From *Eupatorium Ayapana. Journal of Indian Chemical Society*. **66**(4): 286-287.

[14] Sarker, S. D., Gray, A. I. and Waterman, P. G. (1994). Coumarin from *Asterolasia Trymalioides. Journal of Natural Products*, **57** (11): 1549-1551.

[15] Azizi, S.S.S.A., Sukari, M. A., Rahmani, M., Kitajima, M., Aimi, N. and Ahpandi, N. J. (2010). Coumarins from *Murraya paniculata*. *The Malaysian Journal of Analytical Sciences*, **14** (1): 1-5.

[16] Kumar, V., Niyaz, N. M. M., Saminathan, S. and Mahinda Wickramaratne, D. B. (1998). Coumarins from *Paramignya monophylla* Root Bark. *Phytochemistry*. **49** (1): 215-218.

[17] Appendino, G., Tagliapietra, S., Nano, G. M., Picci, V. (1988). Ferprenin, A Prenylated Coumarin from *Ferula Communis*. *Photochemistry*, **27**(3): 944-946.

[18] Pace, R. D. and McWilliams, L. (2006). The Perkin Reaction: Rapid and Efficient Process Optimization through a Microwave/Design of Experiments Couple. *Journal of the Arkansas Academy of Science*, **60**: 96-100.

[19] Kurti, L. and Czako, B. (2005). "*Strategic Applications of Named Reactions in Organic Synthesis*". Elsevier Inc., USA. 242-243, 286-287, 338-339, 472-473, 484-485.

[20] Augustine, J. K., Bombrun, A., Ramappa, B. and Boodappa, C. (2012). An Efficient One-pot Synthesis of Coumarins Mediated by Propylphosphonic Anhydride (T3P) via the Perkin Condensation. *Tetrahedron Letters*, **53**: 4422-4425.

[21] Borges, F., Roleira, F., Milhazes, N., Santana, L. and Uriarte, E. (2005). Simple Coumarins and Analogues in Medicinal Chemistry: Occurrence, Synthesis and Biological Activity. *Current Medicinal Chemistry*. Bentham Science Publisher Ltd. **12**: 887-916.

[22] Zanger, M. and McKee, J. R. (1995). *Small Scale Synthesis: A Laboratory Textbook of Organic Chemistry*. WCB/ McGraw-Hill, USA. 31-45, 52-53, 249-252, 269-272, 429-437.

[23] Kalita, P. and Kumar, R. (2012). Solvent-free Coumarin Synthesis via Pechmann Reaction Using Solid Catalyst. *Microporous and Mesoporous Materials*. **149**: 1-9.

[24] Fang, D., Cheng, J., Gong, K., Shi, Q. and Liu, Z. L. (2008). Synthesis of Coumarins via Pechmann Reaction in Water Catalyzed by Acyclic Acidic Ionic Liquid. *Catalysis Letters.* **121**: 255-259.

[25] Tyagi, Y. K., Tyagi, S., Raj, H. G. and Gupta, R. K. (2008). Synthesis of Novel 4-Methylcoumarins and Comparative Specificities of Substituted Derivatives for Acetoxy Drug: Protein Transacetylase. *Science Pharmacological*. **7**: 395-414.

[26] Mokhtary, M. and Najafizadeh, F. (2012). Polyvinylpolypyrrolidone-bound boron trifluoride (PVPP-BF_3); A Mild and Efficient Catalyst for Synthesis of 4-Methylcoumarins via the Pechmann Reaction. *Comptes Rendus Chimie*, **15**: 530-532.

[27] Bose, D. S., Rudradas, A. P. and Babu, M. H. (2002). The Indium(III) Chloride-Catalyzed Von Pechmann Reaction: A Simple and Effective Procedure for the Synthesis of 4-Substituted Coumarins. *Tetrahedron Letters*, **43**: 9195-9197.

[28] Sinhamahapatra, A., Sutradhar, N., Pahari, S., Bajaj, H. C. and Panda, A. B. (2011). Mesoporous Zirconium Phosphate: An Efficient Catalyst for the Synthesis of Coumarin Derivatives through Pechmann Condensation Reaction. *Applied Catalysis A: General*. **394**: 93-100.

[29] Maghaddam, F. M., Mirjafary, Z. and Saeidian, H. (2009). Microwave-Assisted Synthesis of 3-Substituted Coumarins Using $ZrOCl_3.8H_2O$ as an Effective Catalyst. *Chemistry and Chemical Engineering*. **16**(1): 12-16.

[30] Armstrong, V., Soto, O., Valderrama, J. A. and Tapia, R. (1988). *Synthetic Communication*. **18**: 717–725.

[31] Shaabani, A., Ghadari, R., Rahmati, A. and Rezayan, A. H. (2009). Coumarin Synthesis via Knoevenagel Condensation Reaction in 1,1,3,3,-N,N,N',N'-Tetramethylguanidinium Trifluoroacetate Ionic Liquid. *Journal of the Iranian Chemical Society*. **6**(4): 710-714.

[32] Asiri, A. M. (2003). Synthesis and Characterization of New Coumarin Derivatives as Ultraviolet Absorbers. *Pigment and Resin Technology*. **32**(5): 326-330.

[33] Dittmer, D. C., Li, Q. and Avilov, D. V. (2005). Synthesis of Coumarins, 4-Hydroxycoumarins, and 4-Hydroxyquinolinones by Tekurium-Triggered Cyclisations. *Journal of Organic Chemistry.* **70**: 4682-4686.

[34] Shah, D. N. and Shah, N. M. (1955). The Kostanecki-Robinson Acylation of 5-Hydroxy-6-acetyl-4-methylcoumarin. *Journal of the American Chemical Society*. **77**(8): 1699-1700.

[35] Stahmann, M. A., Wolff, I. and Link, K. P. (1943). Studies on 4-Hydroxycoumarin. I. The Synthesis of 4-Hydroxycoumarins. *Journal of the American Chemical Society*. **65**(12): 2285-2287.

[36] Desai, N. J. and Sethna, S. (1957). Synthesis of Some 4-Hydroxycoumarin Derivatives. *The Journal of Organic Chemistry*. **22**: 388-390.

[37] Zavrsnik, D., Muratovic, S., Spirtovic, S., Softic, D., and Medic-Saric, M. (2008). The Synthesis and Antimicrobial Activity of some 4-Hydroxycoumarin Derivatives. *Bosnian Journal of Basic Medical Sciences*. **8**(3): 277-281.

[38] Jung, J. C. and Park, O. S. (2009). Synthetic Approaches and Biological Activities of 4-Hydroxycoumarin Derivatives. *Molecules*. **14**: 4790-4803.

[39] Sardari, S., Nishibe, S. and Daneshtalab, M. (2000). Coumarins, The Bioactivitie Structures With Antifungal Property. *Studies in Natural Products Chemistry*. Elsevier Science B. V. **23**: 335-393.

[40] Chia, Y. C., Chang, F. R., Wang, J. C., Wu, C. C., Michael Chiang, Y. N., Lan, Y. H., Chen, K. S. and Wu, Y. C. (2008). Antiplatelet Aggregation Coumarins from the Leaves of Murray omphalocarpa. *Molecules*. **13**: 122-128.

[41] Basile, A., Sorbo, S., Spadaro, V,, Bruno, M., Maggio, A., Faraone, N. and Rosselli, S. (2009). Antimicrobial and Antioxidant Activities of Coumarins from the Roots of Ferulago campestris (Apiaceae). *Molecules*. **14**: 939-952.

[42] Lee, S. Y., Lee, Y. S., Jung, S. H., Shin, K. H., Kim, B. K. and Kang, S. S. (2003). Anti-Tumor Activities of Decursinol Angelate and Decursin from *Angelica gigas*. *Archibes of Pharmacal Research*. **26** (9): 727-730.

[43] Céspedes, C. L., Avila, J. G., Martinez, A., Serrato, B., Calderon-Mugica, J. C. and Salgado-Garciglia, R. (2006). Antifungl and Antibacterial Activities of Mexican Tarragon (*Tagetes lucida*). *Journal of Agricultural and Food Chemistry*. **54**: 3521-3527.

[44] Kumar, R., Saha, A., and Saha, D. (2012). A New Antifungal Coumarin from *Clausena excavate*. *Fitoterapia*. **83**: 230-233.

[45] Rodrigo, S. A. A., Felipe, Q. S. G., Edeltrudes, O. L., Carlos, A. S., Josean, F. T., Luciana, S., Marcus, T. S., Thiago, M. A., Ricardo, O. M., Francisco, J. B. M. J., and Barbosa-Filho, J. M. (2013). Synthesis, Structure-Activity Relationships (SAR) and *in Silico* Studies of Coumarin Derivatives with Antifungal Activity. *International Journal of Molecular Sciences*. **14: 1293-1309.**

[46] Munshi,P., Venugopala, K. N., Jayashree, B. S. and Guru Row, T. N. (2004). Concomitant Polymorphism in 3-Acetylcoumarin: Role of Weak C-H ⋯O and C-H⋯π Interactions: *Crystal Growth and Design*. **4**(6): 1105-1107.

[47] Kumar, B. V., Halehatty, S. N., Girija, D. and Vijaya, K. B. (2011). ZnO Nanoparticle as Catalyst for Efficient Green One –pot Synthesis of Coumarin through Knoevenagel Condensation. *Journal of Chemical Sciences.* **123(**5): 615-621.

[48] Vazquez-Rodriguez, S., Serra, S., Santos, Y. and Santana, L. (2010). Efficient Synthesis of Coumarin-Chalcones Hybrids as New Scaffold with Antibacterial Interest. Proceedings of the 14th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-14). 1-30 November 2010.

[49] Pavan Kumar Reddy, C., Mohan Goud, V., Sreenivasulu, N. and Rajendra Prasad (2010). Design, Synthesis and Chemical Characterization of Some Novel Coumarin compounds and Evaluation of their Biological Activity. *International Journal of Phama World Research*. **1** (2): 1-19.

[50] Li, X., Zhao, Y. X., Wang, T., Shi, M. Q. and Wu, F. P. (2006). Coumarin Derivatives with Enhanced Two-Photon Absorption Cross-Sections. *Dyes and Pigments*. **74**: 108-112.

[51] Kumar, T., Dewangan, D., Alexander, A., Nagori, K. and Tripathi, D. K. (2011). Synthesis and Characterization of 8-[(2-Amino-6-aryl-pyrimidinin-4-yl)oxy]-4-methyl-*2H*-Chromen-2-ol Derivatives. *Asian Journal of Biochemical and Pharmaceutical Research*. **1**(2): 393-401.

[52] Yasubumi, M. (2003). Process for Photopolymerization by Exposure of a Photosensitive Lithographic Printing Plate. *European Patent Convention.*

[53] Venothini Appu (2010). *Synthesis of Chalcones and Derivatives.* Master of Science (Chemistry), Universiti Teknologi Malaysia, Skudai.

[54] Bassignana, P. and Cogrossi, C. (1964). Infrared Spectra of certain 3-Acylcoumarin Derivatives. *Tetrahedron.* **20**(12): 2859-2871.

[55] Ng, B. H., Loc, T. B. and Ng, D. X. (1957). New 3-Acylcoumarins. *Bulletin de la Societe Chimique de France. 561-563.*

[56] Al-Bayati, R. I., Al-Amiery, A. A. H. and Al-Majedy, Y. K. (2010). Design, Synthesis and Bioassay of Novel Coumarins. *African Journal of Pure and Applied Chemistry*. **4**(6): 74-86.

[57] Basanagouda, M., Kulkarni, M. V., Sharma, D., Gupta, V. K., Pranesha, Sandhyarani, P. and Rasal, V. P. (2009). Synthesis of some new 4-Aryloxmethylcoumarins and Examination of their Antibacterial and Antifungal Acivities. *Journal of Chemical Sciences*. **121**(4): 485-495.

[58] Thimons, M.; Chua, C. A.; Achalabun, M. (1998). The Pechmann Reaction. *Journal of Chemistry Education*. **75**(12).

[59] Hutchinson, D. W. and Tomlinson, J. A. (1968). The Reaction between Mesityl Oxide and 4-Hydroxycoumarin. *Tetrahedron Letters*. **9**: 5027-5028.

[60] Gasparova, R., Kotlebova, K. and Lacova, M. (2009). Reactions of 4-Hydroxycoumarin with Heterocyclic Aldehydes. *Nova Biotechnologica*. **9**(3): 349-354.

[61] Chakravati and Duhkhaharan (1935). Synthesis of Coumarin from Phenols and β-ketonic esters. Use of various Condensing Agents. *Journal of The Indian Chemical Society.* **12**: 536-539.

[62] Rangaswami, S. and Seshadri, T. R. (1937). Constitution of Coumarinopyrones Derived from 7-Hydroxycoumarins. *Indian Academy of Science.* **6A**: 112-118.

[63] Ramesh, P., Das, A. T., Mohandass, P. and Nagasathya, R. (2008). The Structure of Hantzsch Coumarin. *Indian Journal of Chemistry*. **47B**: 1447-1450.

[64] Bora, U., Chaudhuri, M. K., Dey, D. and Dhar, S. S. (2001). Peroxometal-mediated Envionmentally Favorable Route to Brominating Agents and Protocols for Bromination of Organics. *Pure and Applied Chemistry*. **73**(1): 93-102.

[65] Lawrence Woo, L. W., Purohit, A., Malini, B., Reed, M. J. and Potter, B. V. J. (2000). Potent Active Site-Directed Inhibition of Steroid Sulphatase by Tricyclic Coumarin-Based Sulphamates. *Chemistry and Biology*. **7** (10): 773-791.

[66] Kumar, R. D. and Cheng, C. H. (2002). Novel Cyclization and Reductive Coupling of Bicyclic Olefins with Alkyl Propiolates Catalyzed by Nickel Complexes. *Pure and Applied Chemistry*.**74**(1): 69-75.

[67] Shilin, S. V., Garzad, M. M. and Khilva, V. P. (2008). Synthesis of Dipeptide Derivatives of 3,4-Substituted 7-Hydroxycoumarins. *Chemistry of Natural Compounds*. **44**(3): 301-305.

[68] Sun, G. J., Wang, Z. Y. and Chen, M. H. (2012). Boron Trifluoride Etherate – A Mild and Inexpensive Reagent for Synthesis of Coumarin Derivatives under Solvent Free Conditions. *Advanced Materials Research.* **365**: 228-232.

[69] Dong, X., Chen, Z., Li, M. and Xu, Z. Y. (2012). Synthesis of Coumarin Derivatives under Microwave Irradiation and Solvent Free Condition Catalyzed by HSO_3 Funtionalized Ionic Liquid. *Jingxi Huagong*. **29**(8): 770-773.

[70] Khodabakshi, S. (2012). Barium Dichloride as a Powerful and Inexpensive Catalyst for the Pechmann Condensation without Using Solvent. *International Journal of Organic Chemistry*.

[71] Wade, L.G. (2006). *Organic Chemistry*. 6th Ed. Pearson Education, N. J., Prentice Hall. 623-658.

[72] Guan, A. Y., Liu, C. L., Li, M., Li, Z. N., Zhang, M. X. and Zhang, H. (2011). Synthesis and Bioactivity of Novel Coumarin Derivatives. *Natural Product Communications.* **6**(12): 1917-1920.

[73] Ashish Sethi and Sharma, R. A. (2011). Antioxidant Activity with Total Phenolic Constituents from *Aerva Tomentosa* Forsk. *International Journal of Pharma and Bio Sciences*. **2**(2): 596-603.

[74] Wanasundara, P. K. J. P. D. and Shahidi, F. (2005). *Bailey's Industrial Oil and Fat Products*. 6th Ed. John Wiley & Sons. 431-489.

[75] Kunhan, J. (1976). The Flavanoids. A Class of Semi-Essential Food Components; Their Role in Human Nutrition. *World Review of Nutrition and Dietetics*. **24**: 117-191.

[76] Ramamoorthy, P. K. and Bono, A. (2007). Antioxidant Activity, Total Phenolic and Flavonoid Content of *Morinda citrifolia* Fruit Extracts from Various Extraction Processes. *Journal of Engineering Science and Technology*. **7**(S1): 1-7.

[77] Aqil, F., Ahmed, I. and Mehmood, Z. (2006). Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used India Medicinal Plants. *Turkish Journal of Biology*. **30**: 177-183.

[78] Prior, R. L. (2003). Fruits and Vegetables in the Prevention of Cellular Oxidative Damage. *The American Journal of Clinical Nutrition.* **78**(3): 570-578.

[79] Cai, Y. Z., Luo, Q., Sun, M. and Corke, H. (2004). Antioxidant Activity and Phenolic Compounds of 112 Traditional Chinese Medicinal Plants associated with Anticancer. *Life Sciences*. **74**(17): 2157-2184.

[80] Kaur, C. and Kapoor, H. C. (2002). Anti-oxidant Activity and Total Phenolic Content of some Asian Vegetables. *International Journal of Food and Technology*. **37**: 153-161.

[81] Mccall, M. R. and Frei, B. (1999). Can antioxidant vitamins materially reduce oxidative damage in humans?. *Free radical Biology and Medicine.* **26**: 1034-1053.

[82] Badarinath, A. V., Rao, K. M., Madhu C. S. C., Ramkanth, S., Rajan, T. V. S. and Gnanaprakash, K. (2010). A Review on *In-vitro* Antioxidant Methods: Comparisons, Correlations and Considerations. *International Journal of Pharmaceutical Technology and Research*. **2**(2): 1276-1285.

[83] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition Analysis*. **19**: 669-675.

[84] Huang, D., Ou, B. and Prior, L. R. (2005). The Chemistry behind Antioxidant Capacity Assay. *Journal of Agricultural and Food Chemistry*. **53**: 1841-1856.

[85] Salie, F., Eagles, P. F. K. and Leng, H. M. J. (1996). Preliminary Antimicrobial Screening of Four South African Asteraceae Species. *Journal of Ethnopharmacology*. **52**: 27-33.

[86] Smith, E., Williamson, E., Zloh, M. and Gibbons, S. (2005). Isopimaric Acid from *Pinus nigra* shows Activity against Multidrug-resistant and EMRSA Strains of *Staphylococcus aureus*. Phytotherapy Research. **19**(6): 538-542.

[87] Islam, M. A., Alam, M. M., Choudhury, M. E., Kobayashi, N. and Ahmed, M. U. (2008). Determinationof Minimum Inhibitory Concentration (MIC) of Cloxacillin for Selected Isolates of Methicillin-Resistant Staphylococus aureus (MRSA) with their Antibiogram. *Bangladesh Journal of Veterinary Medicine.* **6**(1): 121-126.

[88] Rios, J. L. and Recio, M. C. (2005). Medicinal Plants and Antimicrobial Activity. *Journal of Ethnopharmacology*. **100**(1-2): 80-84.

[89] Doughari, J. H. (2006). Antimicrobial Activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research*. **5**(2): 597-603.

[90] Marcal, F. J. B., Cortez, D. A. G., Ueda-Nakamura, T., Nakamura, C. V. and Filho, B. P. D. (2010). Activity of the Extracts and Neolignans from *Piper regnellii* against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Molecules*. **15**: 2060-2069.

[91] Abubakar, E. M. (2009). Antibacterial Efficacy of Stem Bark Extracts of *Magifera indica* Against Some Bacteria Associated with Respiratory Tract Infections. *Scientific Research and Essay*. **4**(10): 1031-1037.

[92] Mbaveng, A. T., Ngameni, B., Kuete, V., Simo, I. K., Ambassa, P., Roy, R., Bezabih, M., Etoa, F. X., Ngadjui, B. T., Abegaz, B. M., Meyer, J. J. M., Lall, N. and Beng, V. P. (2008). Antimicrobial Activity of the Crude Extracts and Five Flavonids from Twigs of Dorstenia barteri (Moraceae). *Journal of Ethnopharmacology*. **116**: 483-489.

[93] Pessini, G. L., Filho, B. P. D., Nakamura, C. V. and Cortez, D. A. G. (2003). Antibacterial Activity of Extracts and Neolignans from *Piper regnellii* (Miq) C.DC. var. *pallescens* (C.DC.) Yunck. *Memorias do Instituto Oswaldo Cruz*. **98**: 1115-1120.