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ENZYMATIC SYNTHESES, CHARACTERIZATIONS AND APPLICATIONS OF FATTY HYDROXAMIC ACID DERIVATIVES FROM CANOLA AND PALM OILS

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By

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Chairman: Prof. MD Jelas Haron, PhD

Faculty: Science

Fatty hydroxamic acids and derivatives have attracted many researches due to their pharmaceutical and analytical applications. They have been widely applied as food additives, growth factors, antibacterial agents, fungicides, tumor inhibitors, enzyme inhibitors and metal chelators. A common method for the preparation of hydroxamic acids and their derivatives is via reaction of hydroxylamine or their derivatives with alkyl esters or activated carboxylic acids. However these reactions often contain many steps and require expensive chemicals. In this study, four types of N-substituted fatty hydroxamic acids as the following were synthesized. Phenyl fatty hydroxamic acids (PFHAs), synthesized by reaction of canola oil, palm kernel oil or palm stearin with N-phenyl hydroxylamine (N-PHA). Methyl fatty hydroxamic acids (MFHAs), synthesized by reaction of palm kernel oil and N-methyl hydroxylamine (N-MHA). Isopropyl fatty hydroxamic acids (IPFHAs),

synthesized by reaction of palm kernel oil and N- isopropyl hydroxylamine (N-IPHA). Benzyl fatty hydroxamic acids (BFHAs), synthesized by reaction of palm kernel oil and N-benzyl hydroxylamine (N-BHA). The reactions were carried out in a sealed flask, incubated in water bath shaker at 39°C for 72 hours and catalyzed by immobilized lipase (Lipozyme TL IM or RM IM). The products were then separated by filtration, solvent extraction followed by evaporation of the solvent. The products were characterized by The FT-IR and ^{1}H NMR spectroscopies, CHN elemental analysis and qualitative color test with Cu(II), Fe(III) and V(V) ions. The products as chelating agents were used for extraction of Cu(II) or Fe(III) ions from samples containing mixture of metal ions in aqueous solution. The products and their copper complexes were also evaluated for antibacterial activity on *Escherichia coli (E. Coli)* and *Staphylococcus aureus (S. Aureus)* and antifungal activity on *Candida parapsilosis* (*C. parapsilosis*), *Candida albicans* (*C. albicans*) and *Aspergillus fumigatus* (*A. fumigatus*) using disk and well diffusion methods.

The conversion percentage of phenyl hydroxylaminolysis of the two different commercial canola oils (Ladan and Krystal brands), palm stearin oil and palm kernel oil were 55.6, 52.2, 51.4 and 49.7 %, respectively. In addition the conversion percentage of methyl hydroxylaminolysis, isopropyl hydroxylaminolysis and benzyl hydroxylaminolysis of the commercial palm kernel oil were 77.8, 65.4 and 61.7 %, respectively. The FTIR and ^{1}H NMR spectroscopic data, CHN elemental analysis and qualitative color test on the purified products confirmed the existence of PFHAs, MFHAs, IPFHAs and BFHAs.

The results of metal extraction studies showed that the MFHAs, PFHAs, IPFHAs and BFHAs based on palm kernel oil were able to separate copper (II) ion from mixture of Cu(II) and Mg (II), Ni (II), Al (III), Mn (II) or Co (II) in aqueous media at pH 6.2. A good stripping recovery of Cu(II) was achieved with a single extraction by 3M H_2SO_4. In addition the MFHAs and BFHAs from palm kernel oil were found able to separate iron (III) from a mixture of Fe(III) and Mg(II), Ni(II), Al(III), Mn(II) or Co(II) in aqueous media at pH 1.9. A good stripping recovery of Fe(III) was obtained with a single extraction by 5M HCl.

Antimicrobial study showed that PFHAs and Cu-PFHs had stronger antibacterial properties against *E. coli* compared to *S. aureus*. Also antibacterial property of the PFHAs from canola oil is stronger than palm kernel oil followed by palm stearin. Furthermore the antibacterial properties of the Cu-PFHs from canola oil, palm kernel oil and palm stearin were stronger than antibacterial properties from their PFHAs. Similarly the results also showed that the Cu-PFHs from canola and palm kernel oils have stronger antifungal properties on *C. parapsilosis*, *C. albicans* and *A. fumigatus* compared to their PFHAs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SINTESIS BERENZIM, PENCIRIAN DAN APLIKASI TERBITAN ASID HIDROKSAMIK LEMAK DARIPADA MINYAK KANOLA DAN MINYAK SAWIT

Oleh

HOSSEIN JAHANGIRIAN

Mei 2012

Pengerusi : Prof. MD Jelas Haron, PhD

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Banyak penyelidikan telah dilakukan keatas asid hidroksamik lemak dan terbitannya kerana penggunaannya dalam farmaseutikal dan analisis. Sebatian ini telah digunakan secara meluas sebagai aditif makanan, faktor pertumbuhan, agen anti-bakteria, racun kulat, perencat tumor, perencat enzim dan pengkelat logam. Kaedah biasa untuk penyediaan asid hidroksamik dan terbitannya adalah melalui tindak balas hidroksilamina atau terbitannya dengan alkil ester atau asid karboksilik teraktif. Walau bagaimanapun, tindak balas ini sering mengandungi langkah-langkah yang banyak dan memerlukan bahan kimia yang mahal. Dalam kajian ini, empat jenis asid hidroksamik lemak tertukarganti seperti berikut telah disintesis. Fenil asid hidroksamik lemak (PFHAs), disintesis melalui tindak balas minyak kanola, minyak isirung sawit atau stearin sawit dengan N-fenil hidroksilamina (PHA). Metil asid hidroksamik lemak (MFHAs), disintesis melalui tindak balas

minyak isirung sawit dengan N-metil hidroksilamina (MHA). Isopropyl asid hidroksamik lemak (IPFHAs), disintesis melalui tindak balas minyak isirung sawit dengan N-isopropil hidroksilamina (MHA). Benzil asid hidroksamik lemak(BFHAs), disintesis melalui tindak balas minyak isirung sawit dengan N-benzil hidroksilamina (BHA). Tindak balas telah dijalankan dalam kelalang bertutup yang dieram dalam kukus air berpengaduk pada suhu 39 °C selama 72 jam dengan magkin lipase tersekatgerak (Lipozyme TL IM atau IM RM). Produk kemudiannya dipisahkan melalui penurasan, pengekstrakan pelarut dan diikuti dengan penyejatan pelarut.

Produk telah dicirikan dengan spektroskopi FT-IR dan ^{1}H NMR, analisis unsur CHN dan ujian kualitatif warna dengan ion Cu(II), Fe(III) dan V(V). Produk sebagai agen pengkelat telah digunakan untuk pengekstrakan ion Cu(II) dan Fe(III) daripada sampel larutan akueus yang mengandungi campuran ion logam. Produk dan kompleksnya dengan ion logam kuprum juga dinilai untuk aktiviti anti-bakteria keatas *Escherichia coli (E. Coli)* dan *Staphylococcus aureus (S. aureus)* dan aktiviti antikulat keatas *Candida parapsilosis (C. parapsilosis)*, *Candida albicans (C. albicans)* dan *Aspergillus fumigatus (A. fumigatus)* menggunakan kaedah resapan cakera dan takungan.

Peratus hasil fenil-hidroksilaminolilis minyak kanola (jenama Ladan dan Krystal), stearin sawit dan minyak isirung sawit masing-masing adalah 55.6, 52.2, 51.4 dan 49.7%. Di samping itu, peratus hasil metil-hidroksilaminolisis, isopropil-hidroksilaminolisis dan benzil-hidroksilaminolisis minyak isirung sawit komersial masing-masing adalah 77.8, 65.4 dan 61.7%. Data spektroskopi FTIR dan ^{1}H NMR

, analisis unsur CHN dan ujian kualitatif warna keatas produk yang telah ditulenkan mengesahkan kewujudan PFHAs, MFHAs, IPFHAs dan BFHAs.

Keputusan kajian pengekstrakan ion logam menunjukkan bahawa MFHAs, PFHAs, IPFHAs dan BFHAs daripada minyak isirung kelapa sawit dapat memisahkan ion Cu(II) dari campuran Cu(II) dengan Mg(II), Ni(II), Al(III), Mn(II) atau Co (II) dalam larutan akueus pada pH 6.2. Pelucutan Cu(II) telah dicapai degan sekali pengekstrakan semula menggunakan H_2SO_4 3M. Di samping itu MFHAs dan BFHAs daripada minyak isirong sawit didapati mampu untuk memisahkan Fe(III) daripad larutan akueus yang mengandungi campuran Fe (III) dengan Mg(II), Ni(II), Al(III), Mn(II) atau Co(II) pada pH 1.9. Pelucutan Fe(III) telah dicapai melalui pengekstrakan semula dengan HCl 5M.

Kajian antimikrobial menunjukkan bahawa PFHAs dan Cu-PFHs mempunyai sifat antibakteria yang kuat terhadap *E. coli* berbanding *S. aureus*. Juga sifat antibakteria PFHAs daripada minyak kanola adalah lebih kuat daripada PFHAs minyak isirung sawit diikuti oleh PFHAs stearin sawit. Sebagai tambahan, sifat antibakteria Cu-PFHs daripada minyak kanola, minyak isirung sawit dan stearin sawit adalah lebih kuat berbanding PFHAs masing-masing. Keputusan kajian juga menunjukkan bahawa Cu-PFHs daripada minyak kanola dan isirong sawit mempunyai sifat antikulat yang kuat keatas *C. parapsilosis*, *C. albicans* dan *A. fumigatus* berbanding PFHAs masing-masing.

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I certify that Examination Committee has met on date of viva voce to conduct the final examination of Hossein Jahangirian on his Degree of Doctor of Philosophy thesis entitled "Enzymatic syntheses, characterizations and applications of fatty hydroxamic acid derivatives based on canola and palm oils" in accordance with University and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Doctor of Philosophy.

Member of the Examination committee were as follows

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and it is not concurrently, submitted for any other degree at University Putra Malaysia or at any institutions.

HOSSEIN JAHANGIRIAN

Date: 25 May 2012

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