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สารสกัดเอนไซม์จาก *Jorunna funebris*



นางสาวดาลัด วโรภาสตระกูล



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต  
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DETECTION OF ESTERASE ACTIVITY CONVERTING RENIERAMYCIN M TO  
JORUNNAMYCIN A IN THE CRUDE ENZYME EXTRACTS OF *JORUNNA FUNEBRIS*

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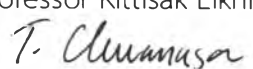
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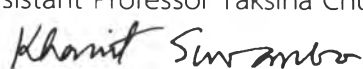
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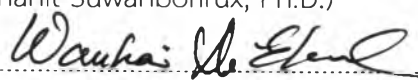
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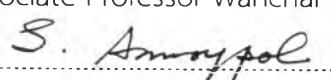
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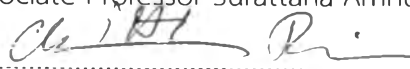
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โจร์นนามัยซินเอ เป็นสารแอลคาลอยด์ในกลุ่มบิสเตตราไฮโดรไอโซควิโนลินควิโนน ซึ่งถูกนำมาใช้เป็นสารตัวกลางเพื่อสังเคราะห์สารอนุพันธ์ 22-O-acyl ของสารเรเนียร์รามัยซินเอ็ม ในการเตรียมสารโจร์นนามัยซินเอทำได้โดยกระบวนการทางเคมีเพื่อตัดหมู่ 22-O-angeloyl ของสารเรเนียร์รามัยซินเอ็ม นอกจากนี้ยังสามารถแยกสารโจร์นนามัยซินเอ ได้จากหากเปลือย *Jorunna funebris*

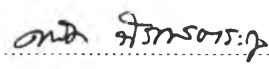
ได้ศึกษาคุณสมบัติในการเปลี่ยนสารเรเนียร์รามัยซินเอ็มเป็นสารโจร์นนามัยซินเอ ของสารสกัดหยาบเอนไซม์ที่เตรียมได้จากเนื้อเยื่ออวัยวะภายในของ *J. funebris* ทำให้เชื่อว่ามีเอนไซม์เอสเทอร์เอส ที่สามารถเร่งปฏิกิริยาไฮโดรลิซิสในการตัดพันธะเอสเทอร์ หมู่ 22-O-angeloyl ของสารเรเนียร์รามัยซินเอ็มได้ และพบว่าเอนไซม์นี้ตกตะกอนด้วยแอมโมเนียมซัลเฟต ในช่วงความเข้มข้นร้อยละ ๕๕ - ๗๕ จากการศึกษาครั้งนี้พบว่าสารสกัดเอนไซม์สามารถทำงานได้ดีที่สุดเมื่อใช้สารละลายบัฟเฟอร์ Tricine ที่อุณหภูมิ ๒๕ องศาเซลเซียส ภายใต้สภาวะต่างที่พีเอช ๘ รวมทั้งพบว่าสารสกัดหยาบเอนไซม์มีความจำเพาะต่อโครงสร้างของสารกลุ่มเรเนียร์รามัยซิน ดังนี้ ๑) คาร์บอนตำแหน่งที่ ๑๕ และ ๑๘ ต้องเป็นหมู่ควิโนน ๒) คาร์บอนตำแหน่งที่ ๑๔ ไม่ควรมีหมู่แทนที่ออกซิเจน ๓) พันธะคู่ที่คาร์บอนตำแหน่งที่ ๒๕ เป็นแบบทรานส์ และ ๔) หมู่ 22-O-aliphatic acyl ที่มีจำนวนมากกว่า ๕ คาร์บอนมีความจำเพาะน้อยกว่าหมู่ที่มีจำนวนคาร์บอนน้อย

ผลการศึกษานี้เป็นการรายงานครั้งแรกเพื่อเป็นข้อมูลในการนำเอนไซม์ที่ได้จากหากเปลือย *J. funebris* ซึ่งเป็นเอนไซม์ที่มีความจำเพาะต่อสารเรเนียร์รามัยซินเอ็ม สามารถนำมาพัฒนาใช้ในการเตรียมสารโจร์นนามัยซินเอ แทนกระบวนการสังเคราะห์ทางเคมี


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DALAD WAROPASTRAKUL: DETECTION OF ESTERASE ACTIVITY  
CONVERTING RENIERAMYCIN M TO JORUNNAMYCIN A IN THE CRUDE  
ENZYME EXTRACTS OF *JORUNNA FUNEBRIS*. ADVISOR: ASST. PROF.  
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JorunnamycinA, a member of the *bis*-tetrahydroisoquinolinequinone  
alkaloids, has been recently used as an important starting material to prepare  
various 22-*O*-acyl derivatives of renieramycin M. It was synthesized by chemical  
reactions to remove the 22-*O*-angeloyl of renieramycin M. Besides, jorunnamycin  
A was recently isolated from a marine nudibranch *Jorunna funebris*.

Our preliminary result suggested that the crude enzyme extract prepared  
from visceral organs of *J. funebris* possibly contained the esterase enzyme. The  
enzyme extract was able to convert renieramycin M to jorunnamycin A by  
exhibiting the catalytic activity for ester bond hydrolysis of the 22-*O*-angeoyl  
moiety of renieramycin M. The enzyme was suitably precipitated by ammonium  
sulfate at the concentrations of 55-75%. According to our study, the optimal  
condition for the enzyme activity was performed with Tricine buffer at 45°C and  
pH 8. Additionally, the crude enzyme showed specificity to the renieramycin  
structures as follows: i) C-15 and C-18 must be a quinone moiety, ii) C-14 should  
not contain an oxygenated substituent, iii) the *trans* double bond geometry at C-  
25 is more preferable than the *cis*, and iv) the 22-*O*-aliphatic acyl group containing  
more than 5 carbons is less favorable than the shorter chain.

This is the first study to report the information of the esterase enzyme  
from the nudibranch *J. funebris* which is highly specific to the substrate  
renieramycin M. The enzyme will be useful as an alternative approach replacing  
the chemical reactions to prepare jorunnamycin A.

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