

**ลำดับกรดอะมิโนและฤทธิ์ทางชีวภาพของโปรตีนจากดอกแคบ้าน *Sesbania grandiflora***

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**AMINO ACID SEQUENCES AND BIOLOGICAL ACTIVITIES  
OF PROTEINS FROM *Sesbania grandiflora* FLOWERS**

**Miss Apaporn Boonmee**

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**Thesis Advisor** Associate Professor Polkit Sangvanich, Ph.D.

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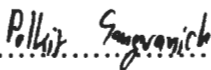


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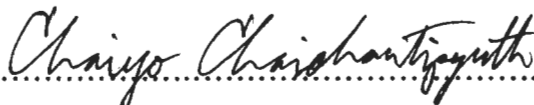
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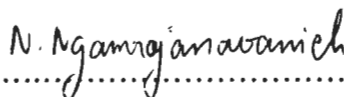
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โปรตีนจากพืชตระกูลถั่วชนิดต่างๆนอกจากจะมีคุณค่าทางโภชนาการแล้วโปรตีนบางชนิด  
 ยังมีฤทธิ์ทางชีวภาพและสามารถนำไปประยุกต์ใช้ให้เกิดประโยชน์ได้ในหลายด้าน ดังนั้นงานวิจัย  
 นี้จึงสนใจที่จะศึกษาลำดับกรดอะมิโนและฤทธิ์ทางชีวภาพของโปรตีนจากดอกแคบ้าน (*Sesbania  
 grandiflora* (L.) Desv.) ซึ่งเป็นพืชตระกูลถั่วที่มีข้อมูลงานวิจัยเกี่ยวกับโปรตีนค่อนข้างน้อย โดย  
 นำดอกแคบ้านมาสกัดและตกตะกอน โปรตีนด้วยเกลือแอมโมเนียมซัลเฟตจากนั้นนำตะกอน  
 โปรตีนไปแยกโดยใช้เทคนิคไอออนเอ็กซ์เชนจ์และ เจลฟิลเทรชันโครมาโทกราฟี โปรตีนที่ได้ คือ  
 SGF60 และ SGF90 มวลโมเลกุลเท่ากับ 40 กิโลดาลตัน และ 63 กิโลดาลตัน ตามลำดับ โปรตีน  
 ทั้งสองชนิดนี้สามารถยับยั้งเอนไซม์แอลฟาไกลูโคซิเดสและมีฤทธิ์ทำให้เม็ดเลือดแดงของกระต่าย  
 เกาะตัวกันได้จากการวิเคราะห์ลำดับกรดอะมิโนของโปรตีนด้วยเทคนิคแทนเดมแมสสเปกโตร  
 เมตรีแบบ ESI-Q-TOF พบว่าโปรตีน SGF60 มีลำดับกรดอะมิโนบางส่วนที่คล้ายคลึงกับโปรตีน  
 p27SJ ซึ่งมีฤทธิ์ยับยั้งเชื้อ HIV-1 ที่ได้จาก *Hypericum perforatum* ส่วนโปรตีนSGF90  
 มีลำดับกรดอะมิโนบางส่วนคล้ายคลึงกับโปรตีนbeta-glucosidase (At5g36890) และ  
 beta-glucosidase F8K4.3 ที่ได้จาก *Arabidopsis thaliana* นอกจากนี้ยังใช้เทคนิคเจลอิเล็กโทร  
 โฟเรซิสแบบสองมิติแยกโปรตีนจากดอกแคบ้านพบว่ามีโปรตีนอย่างน้อย 6 ชนิดเป็นองค์ประกอบ

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....อภาพร บุญมี.....  
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KEY WORD: *Sesbania grandiflora*/ LEGUME/ PROTEIN/  $\alpha$ -GLUCOSIDASE  
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APAPORN BOONMEE: AMINO ACID SEQUENCE AND BIOLOGICAL  
ACTIVITIES OF PROTEINS FROM *Sesbania grandiflora* FLOWERS.

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Legume proteins present a lot of nutrition value for human consumption. Some of them are bioactive proteins which can be application to many works. Protein from *Sesbania grandiflora* (L.) Desv., leguminous tree, has a few records in databases. Therefore, this research interested to study composition and biological activities of proteins from *Sesbania grandiflora* flowers. Crude Proteins from *Sesbania grandiflora* flowers were extracted and fractionation precipitated with ammonium sulfate. By using ion exchange and gel filtration chromatography, SGF60 and SGF90 were separated. Both purified proteins show  $\alpha$ -glucosidase inhibitory activity and hemagglutinating activity with rabbit erythrocyte. Approximate molecular weight of SGF60 and SGF90 protein from gel electrophoresis are 40 and 63 kDa, respectively. When analyzed amino acid sequence of these proteins by using ESI-Q/TOF, the result shown that this SGF60 has partial of amino acid sequence similar to p27SJ, a novel protein inhibited HIV-1, from *Hypericum perforatum*. For SGF90, the amino acid sequences of this protein matched with beta-glucosidase (At5g36890) and beta-glucosidase F8K4.3 protein from *Arabidopsis thaliana*. Moreover, 2-D gel electrophoresis was used to separate crude protein precipitating with TCA/acetone solution. From this technique, there are at less six proteins in *Sesbania grandiflora* flowers.

Department.....Chemistry.....Student's signature..... *Apaporn Boonmee*  
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**LIST OF ABBREVIATIONS**

app	Approximate
ACN	Acetonitrile
AGI	Alpha glucosidase inhibition
APS	Ammonium persulfate
BSA	Bovine serum albumin
cal	Calculation
CCA	$\alpha$ -Cyano-4-hydroxycinnamic acid
°C	degree Celsius
cm	centimeters
CP	Crude protein
CM	Carboxymethyl
CID	collision-induced dissociation
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DTT	Dithiothreitol
2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
DC	Direct current
DEAE	Diethylaminoethyl
ESI	Electrospray ionization
EtOH	Etanol
EDTA	Ethylenediaminetetraacetic acid
g	gram
h	hour
HIV	human immunodeficiency virus
HPLC	High performance liquid chromatography
HU	Hemagglutinating unit
IAA	iodoacetamide
IPG	Immobilized pH gradients
IEF	Isoelectric focusing

kDa	kilo Dalton
kVh	kilo volt-hour
kg	kilogram
μl	microliter
MW	Molecular weight
MS	Mass spectrometry
MS-MS	Tandem Mass spectrometry
MALDI	Matrix Assisted Laser Desorption Ionization
mM	millimolar
mA	milliampere
ml	milliliter
mm	millimeter
mg	milligram
min	minute
m/z	mass per charge ratio
nm	nanometer
No	Number
NCBI	National Center of Biotechnology Information
Native PAGE	Non-denaturing polyacrylamide gel electrophoresis
OD	Optical density
ob	observe
pI	Isoelectric point
ppm	part per million
PNPG	<i>p</i> -nitrophenyl - $\alpha$ -D-glucopyranoside
PMF	Peptide mass fingerprint
Q	Quaternary ammonium
RBC	Red blood cell
RF	Radio frequency
RPC	Reverse phase chromatography
RP-HPLC	Reverse phase High performance liquid chromatography
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate

<b>SDS-PAGE</b>	<b>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</b>
<b>TEMED</b>	<b><i>N,N,N',N'</i>-tetramethylethylenediamine</b>
<b>TOF</b>	<b>Time of flight</b>
<b>TCA</b>	<b>Trichloro acetic acid</b>
<b>Tris</b>	<b>Tris(hydroxymethy)-aminoethane</b>
<b>TFA</b>	<b>Trifluoro acetic acid</b>
<b>U</b>	<b>Unit</b>
<b>V</b>	<b>Volt</b>
<b>v/v</b>	<b>volume by volume</b>
<b>ZMB</b>	<b>Zero moisture basis</b>