

ฤทธิ์ต้านออกซิเดชันจากว่านม้าเหลือง *Curcuma* spp.



นางสาววิไลวรรณ มโนกวินโชค

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ANTIOXIDANT ACTIVITY FROM *Curcuma* spp.

Miss Wilaiwan Manokawinchoke

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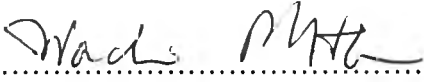
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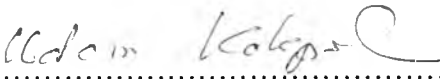
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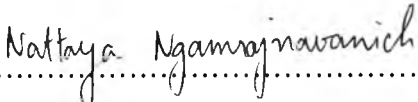
  
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วิไลวรรณ มโนกวินโชค : ฤทธิ์ต้านออกซิเดชันจากว่านม้าเหลือง *Curcuma* spp.  
(ANTIOXIDANT ACTIVITY FROM *Curcuma* spp.) อ. ที่ปรึกษา : ผศ.ดร. สันติ ทิพยางค์,  
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จากการทดสอบฤทธิ์ต้านออกซิเดชันเบื้องต้นจากพืชในวงศ์ Zingiberaceae พบว่า สิ่งสกัด  
ไดคลอโรมีเทนและสิ่งสกัดเอธิลอะซีเตตจากว่านม้าเหลืองมีฤทธิ์ที่ดีต่อการต้านอนุมูล 2,2-diphenyl-1-  
picrylhydrazyl (DPPH) ซึ่งเป็นอนุมูลอิสระที่มีความเสถียร เมื่อนำสิ่งสกัดเหล่านี้มาทำการแยกพบ  
ว่า สามารถแยกสารได้ 5 ชนิดได้แก่ curcumin (1), demethoxycurcumin (2), bisdemethoxy  
curcumin (3), *ar*-turmerone (4), และ 1-hydroxy-1,2-di-(6-methyl-3-isopropenyl-2-propionyl  
oxy-1-cyclohexene)-1-propene (5) การหาสูตรโครงสร้างของสารทั้งหมดหาได้โดยใช้วิธีการทาง  
สเปคโตรสโคปีและเปรียบเทียบกับข้อมูลที่ได้มีการรายงานไว้แล้ว สำหรับฤทธิ์ต้านออกซิเดชันของสาร  
ที่แยกได้มีวิธีการทดสอบคือ วิธีทดสอบฤทธิ์ต้านอนุมูล DPPH วิธีทดสอบฤทธิ์ที่เกี่ยวข้องกับเอนไซม์  
xanthine oxidase (ฤทธิ์ต้านอนุมูล superoxide และ ฤทธิ์ยับยั้งเอนไซม์ xanthine oxidase) และ  
วิธีทดสอบการยับยั้งการเกิดออกซิเดชันในไขมันโดยเปรียบเทียบกับ BHA และ allopurinol จากผล  
การทดสอบฤทธิ์ต้านอนุมูล DPPH พบว่า สาร 1, 2, 4 และ 5 แสดงฤทธิ์ได้ดีโดยมี  $IC_{50} = 0.16, 0.27,$   
 $0.17$  และ  $0.19$  mM ตามลำดับ สาร 3 แสดงฤทธิ์ต่ำที่สุด ( $IC_{50} = >0.50$  mM) ส่วนฤทธิ์ต้านอนุมูล  
superoxide นั้นเรียงตามลำดับได้ดังนี้ สาร 1 ( $IC_{50} = 0.31$  mM), สาร 2 ( $IC_{50} = 0.33$  mM), สาร 4  
( $IC_{50} = 0.34$  mM), สาร 3 ( $IC_{50} = 0.35$  mM), และ สาร 5 ( $IC_{50} = 0.36$  mM) นอกจากนี้สารทั้งหมด  
ยังแสดงฤทธิ์ยับยั้งเอนไซม์ xanthine oxidase และการเกิดออกซิเดชันในไขมันได้สูงอีกด้วยโดยเฉพาะ  
สาร 1 ( $IC_{50} = 0.30$  และ  $0.19$  mM) รองลงมาคือ สาร 4 ( $IC_{50} = 0.30$  และ  $0.21$  mM), สาร 2  
( $IC_{50} = 0.31$  และ  $0.22$  mM), สาร 5 ( $IC_{50} = 0.31$  และ  $0.24$  mM), และสาร 3 ( $IC_{50} = 0.42$  และ  
 $0.27$  mM)

หลักสูตร.....เทคโนโลยีชีวภาพ..... ลายมือชื่อนิสิต..... วิไลวรรณ มโนกวินโชค  
สาขาวิชา.....เทคโนโลยีชีวภาพ..... ลายมือชื่ออาจารย์ที่ปรึกษา..... สันติ ทิพยางค์  
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WILAIWAN MANOKAWINCHOKE: ANTIOXIDANT ACTIVITY FROM *Curcuma* spp.

THESIS ADVISOR :ASST. PROF. Dr. SANTI TIP-PYANG, 88 pp. ISBN 974-17-1966-3.

In the course of screening for antioxidant activity from plants in Zingiberaceae family, the dichloromethane and ethyl acetate crude extracts from *Curcuma* spp. (Waan Ma Lueang) were found to have a promising activity, as guided by scavenging effect on the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). These crude extracts were separated and led to the isolation of five compounds, namely, curcumin (1), demethoxycurcumin (2), and bisdemethoxy curcumin (3), *ar*-turmerone (4), and 1-hydroxy-1,2-di-(6-methyl-3-isopropenyl-2-propionyloxy-1-cyclohexene)-1-propene (5). The structures of all compounds were elucidated on the basis of spectroscopic methods, as well as comparison with literature data. In terms of antioxidant activity, the isolated compounds were evaluated by various *in vitro* model assays, which include the DPPH radical scavenging activity, xanthine oxidase-related activity (superoxide scavenging activity and inhibitory effect on xanthine oxidase), and lipid peroxidation inhibitory activity, by comparison with BHA and allopurinol. The free radical scavenging activity on DPPH indicated that compounds 1, 2, 4, and 5 exhibited significant activity with  $IC_{50} = 0.16, 0.27, 0.17,$  and  $0.19$  mM, respectively, while compound 3 gave the weakest activity ( $IC_{50} = >0.50$  mM). Their superoxide scavenging activity was in order of compound 1 ( $IC_{50} = 0.31$  mM), 2 ( $IC_{50} = 0.33$  mM), 4 ( $IC_{50} = 0.34$  mM), 3 ( $IC_{50} = 0.35$  mM), 5 ( $IC_{50} = 0.36$  mM). Furthermore, all compounds displayed highly inhibitory activity against xanthine oxidase and lipid peroxidation, particularly compound 1 ( $IC_{50} = 0.30$  and  $0.19$  mM), followed by compound 4 ( $IC_{50} = 0.30$  and  $0.21$  mM), 2 ( $IC_{50} = 0.31$  and  $0.22$  mM), 5 ( $IC_{50} = 0.31$  and  $0.24$  mM), and 3 ( $IC_{50} = 0.42$  and  $0.27$  mM), respectively.

Program of.....Biotechnology..... Student's signature...*Wilaiwan Manokawinchoke*.....  
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### List of abbreviations

$^{13}\text{C}$ NMR	= carbon 13 nuclear magnetic resonance
$^1\text{H}$ NMR	= proton nuclear magnetic resonance
ATP	= adenosine triphosphate
BHA	= butylated hydroxyanisole
BHT	= butylated hydroxytoluene
$^{\circ}\text{C}$	= degree celsius
CoQ <sub>10</sub>	= ubiquinone
COSY	= two-dimension $^1\text{H}$ correlation spectroscopy
CD <sub>3</sub> COOCD <sub>3</sub>	= deuterated acetone
CDCl <sub>3</sub>	= deuterated chloroform
CH <sub>2</sub> Cl <sub>2</sub>	= dichloromethane
CHCl <sub>3</sub>	= chloroform
d	= doublet
dd	= doublet of doublet
DEPT	= distortionless enhancement by polarization transfer
DPPH	= 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl
DMSO	= dimethylsulfoxide
EC. 1.2.3.2	
EC	= enzyme code
1	= Oxidoreductase (enzyme class), oxidation/reduction reactions
2	= Aldehyde or ketone (electron donator)
3	= Oxygen (electron acceptor)
EtOAc	= ethyl acetate
EIMS	= electron impact mass spectrometry
FT	= fourier transform
FTC	= ferric thiocyanate
g	= gram
HMBC	= heteronuclear multiple bond connectivity by 2D multiple quantum NMR

**List of abbreviations (continued)**

HMQC	= <sup>1</sup> H-detected heteronuclear multiple quantum coherence via direct coupling
Hz	= hertz
IC <sub>50</sub>	= inhibition concentration at 50%
IR	= infrared
<i>J</i>	= coupling constant
M	= molar
MeOH	= methanol
m	= multiplet
m.p.	= melting point
mM	= millimolar
mg	= milligram
ml	= milliliter
mm	= millimeter
m/z	= mass per charge
No.	= number
nm	= nanometer
ppm	= parts per million
q	= quartet
ROS	= reactive oxygen species
R <sub>f</sub>	= retardation factor
SDS	= sodium dodecylsulfate
SiO <sub>2</sub>	= silica gel
s	= singlet
spp.	= species
<i>t</i>	= trans
TBHQ	= tertiary butylhydroquinone
v/v	= volume by volume
w/v	= weight by volume



**List of abbreviations (continued)**

w/w	= weight by weight
XOD	= xanthine oxidase
$\delta$	= chemical shift
$\lambda_{\max}$	= maximum wavelength
$\nu_{\max}$	= wavenumber cause maximum absorption