

สารประกอบที่มีฤทธิ์คล้ายเอสโตรเจนจากเหง้าว่านหางจิ้งและแก่นครี



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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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ESTROGENIC-LIKE COMPOUNDS FROM *BELAMCANDA CHAINENSIS* RHIZOMES AND
DALBERGIA PARVIFLORA HEARTWOOD

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Belamcanda chinensis (L.) DC (Iridaceae) และ *Dalbergia parviflora* Roxb. (Leguminosae) เป็นพืชสองชนิดที่พบว่ามีฤทธิ์คล้ายฮอร์โมนเอสโตรเจนในการทดสอบขั้นคัดกรอง การศึกษาทางพฤกษเคมีของว่านหางช้าง สามารถแยกสารใหม่ในกลุ่ม phenolic ได้ 3 ชนิด คือ belamphenone belalloside A และ belalloside B นอกจากนี้ยังพบสารที่มีรายงานมาแล้วได้อีก 13 ชนิด ได้แก่ tectorigenin irisfloreantin irigenin irilin D tectoridin iristectorin B iristectorin A iridin hispiduloside jaceoside androsin iriflophenone และ resveratrol สำหรับการศึกษาด้านพฤกษเคมีของแก่นครี สามารถแยกสารได้ 41 ชนิด ซึ่งเป็นสารใหม่ 9 ชนิด ได้แก่ สารกลุ่ม isoflavones 5 ชนิด (khrinone A-E) isoflavan 1 ชนิด (khrinol A) isoflavanone 1 ชนิด (dalparvin) และสารกลุ่ม dihydroflavonol 2 ชนิด (dalparvinol A และ dalparvinol B) นอกจากนี้ ยังพบสารกลุ่ม flavonoid ที่มีรายงานมาแล้วอีก 32 ชนิด ได้แก่ mucronulatol 7-demethylrobustigenin 3'-methoxyviolanonone onogenin sativanone pinocembrin biochanin A hydroxyobtustystyrene 2'-methoxybiochanin A (6a, 11a)-3,8-dihydroxy-9-methoxypterocarpan 8-demethylduartin pinobanksin secundiflorol H 7,3'-dihydroxy-4'-methoxyisoflavanone violanonone arizonicanol A tectorigenin vestitone pratensein 2'-methoxyformononetin formononetin vestitol xenognosin 5'-methoxyvestitol 3'-methoxydaidzein calycosin theralin naringenin genistein liquiritigenin isoliquiritigenin และ bowdichione การพิสูจน์สูตรโครงสร้างทางเคมีของสารที่แยกได้นี้อาศัยการวิเคราะห์สเปกตรัมของ UV, MS, NMR ร่วมกับการเปรียบเทียบข้อมูลของสารที่ทราบโครงสร้างแล้ว นอกจากนี้ยังได้นำสารที่แยกได้ไปทดสอบฤทธิ์คล้ายฮอร์โมนเอสโตรเจน พบว่า สารกลุ่ม flavonoid เกือบทั้งหมดมีฤทธิ์สูงในการเพิ่มจำนวนเซลล์ MCF-7 และ T47D อีกทั้งยังมีฤทธิ์กระตุ้นการแสดงออกของยีนส์ luciferase ในเซลล์ MCF-7/Luc และ T47D/Luc โดยพบว่า genistein, formononetin, khrinone D, biochaninA, theralin, naringenin, liquiritigenin, (6a,11a)-3,8-dihydroxy-9-methoxypterocarpan, isoliquiritigenin และ xenognosin มีฤทธิ์กระตุ้นการแสดงออกของยีนส์ luciferase สูงที่สุด ผลการศึกษานี้สนับสนุนการใช้พืชสมุนไพรทั้งสองชนิดในการรักษาโรคที่เกิดจากความผิดปกติทางฮอร์โมน

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Belamcanda chinensis (L.) DC (Iridaceae) and *Dalbergia parviflora* Roxb. (Leguminosae) are two plant species that appeared to have strong estrogenic-like activity based on our preliminary screening test. Subsequent phytochemical studies of the rhizomes of *Belamcanda chinensis* led to isolation of three new phenolics and 13 known compounds. The three new phenolics were named: belamphenone, belalloside A and belalloside B and the known compounds were: tectorigenin, irisfloreantin, irigenin, irilin D, tectoridin, iristectorin B, iristectorin A, iridin, hispiduloside, jaceoside, androsin, iriflophenone and resveratrol. For *Dalbergia parviflora*, 41 compounds were isolated from its heartwood. Among these, 9 compounds appeared to be new, including 5 isoflavones which were named khrinone A-E; a new isoflavan, khriol A; a new isoflavanone, dalparvin; and two dihydroflavonols, dalparvinol A and dalparvinol B. For the 32 known flavonoids, these were found to be: mucronulatol, 7-demethylrobustigenin, 3'-methoxyviolanonone, onogenin, sativanone, pinocembrin, biochanin A, hydroxyobtustyrene, 2'-methoxybiochanin A, (6a, 11a)-3,8-dihydroxy-9-methoxypterocarpan, 8-demethylduartin, pinobanksin, secundiflorol H, 7,3'-dihydroxy-4'-methoxyisoflavanone, violanonone, arizonicanol A, tectorigenin, vestitone, pratensein, 2'-methoxyformononetin, formononetin, vestitol, xenognosin, 5'-methoxyvestitol, 3'-methoxydaidzein, calycosin, theralin, naringenin, genistein, liquiritigenin, isoliquiritigenin, and bowdichione. Structure elucidations of all the isolates were accomplished by various spectroscopic methods, including comparison of their UV, MS, NMR properties previously reported for the known compounds. Finally, all the isolated compounds were evaluated for their estrogenic activities. It was found that most of them showed strong stimulatory activities on cell proliferation for both MCF-7 and T47D cells. Among these, high luciferase inducing activity in both MCF-7/Luc and T47D/Luc cells were observed for genistein, formononetin, khrinone D, biochanin A, theralin, naringenin, liquiritigenin, (6a, 11a)-3,8-dihydroxy-9-methoxypterocarpan, isoliquiritigenin and xenognosin. These results support the traditional use of *Belamcanda chinensis* and *Dalbergia parviflora* as the treatment of hormonal disorders.

Field of study Pharmaceutical Chemistry
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LIST OF ABBREVIATIONS AND SYMBOLS

α	=	Alpha
β	=	Beta
δ	=	Chemical shift
ϵ	=	Molar absorptivity
$^{\circ}\text{C}$	=	Degree Celsius
μg	=	Microgram
μL	=	Microliter
μM	=	Micromolar
λ_{max}	=	Wavelength at maximal absorption
$[\alpha]^{25}_{\text{D}}$	=	Specific rotation at 25 $^{\circ}\text{C}$ and sodium D line (589 nm)
$[\text{M}+\text{H}]^{+}$	=	Protonated molecule
$[\text{M}+\text{Na}]^{+}$	=	Sodium adduct ion
^{13}C NMR	=	Carbon-13 Nuclear Magnetic Resonance
1D	=	One Dimensional
^1H NMR	=	Proton Nuclear Magnetic Resonance
^1H - ^1H COSY	=	Homonuclear (Proton-Proton) Correlation Spectroscopy
2D	=	Two Dimension
4CL	=	4-coumaroyl:CoA-ligase
4H	=	Cinnamate – 4 – hydroxylase
Acetone- d_6	=	Deuterated acetone
Api	=	Apiose
br	=	Broad
C4H	=	Cinnamate-4-hydroxylase
Calcd	=	Calculated
CD	=	Circular Dichroism
$\text{CD}_3\text{CO CD}_3$	=	Deuterated acetone
CD_3OD	=	Deuterated methanol
CDCl_3	=	Deuterated chloroform
CH_3CN	=	Acetonitrile
CHCl_3	=	Chloroform
CHI	=	Chalcone isomerase

CHR	=	Chalcone reductase
CHS	=	Chalcone synthase
Cm	=	Centimeter
CO ₂	=	Carbon dioxide
d	=	Doublet (for NMR spectra)
DCC	=	Dextran-coated charcoal
dd	=	Doublet of doublets (for NMR spectra)
ddd	=	Doublet of doublet of doublet (for NMR spectra)
DFR	=	Dihydroflavonol-4-reductase
DMID	=	7,2'-dihydroxy-4'-methoxyisoflavanol dehydratase
DMSO	=	Dimethyl sulfoxide
DMSO- <i>d</i> ₆	=	Deuterated dimethyl sulfoxide
E ₂	=	17β-Estradiol
ED ₅₀	=	50% Effective Dose
EDTA	=	Diaminoethane tetraacetic acid
EIMS	=	Electron Impact Mass Spectrometry
EqE ₁₀	=	Stimulated cell proliferation concentration equivalent to 10 pM estradiol
EqE ₁₀₀	=	Stimulated cell proliferation concentration equivalent to 100 pM estradiol
ER	=	Estrogen receptor
ERE	=	Estrogen response element
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
F3'5'H	=	Flavonoid-3',5'-hydroxylase
F3'H	=	Flavonoid-3'-hydroxylase
F3H	=	Flavanone-3-hydroxylase
FABMS	=	Fast Atom Bombardment Mass Spectrometry
FBS	=	Fetal bovine serum
Fr.	=	Fraction
FSI and FSII	=	Flavone synthase
g	=	Gram
Gal	=	Galactose

GC	=	Gas Liquid Chromatography
Glc UA	=	Glucuronic acid
Glc	=	Glucose
H ₂ O	=	Water
HMBC	=	¹ H-detected Heteronuclear Multiple Bond Coherence
HMQC	=	¹ H-detected Heteronuclear Multiple Quantum Coherence
HPLC	=	High Performance Liquid Chromatography
hr	=	Hour
HRFABMS	=	High Resolution Fast Atom Bombardment Mass Spectrometry
Hz	=	Hertz
I2'H	=	Isoflavone-2'-hydroxylase
I6H	=	Isoflavone-6-hydroxylase
IC ₅₀	=	Median Inhibitory Concentration
IFR	=	Isoflavone reductase
IFS	=	Isoflavone synthase
IOMT	=	Isoflavone- <i>O</i> -methyltransferase
<i>J</i>	=	Coupling constant
Kg	=	Kilogram
L	=	Litter
Luc	=	Luciferase gene
<i>m</i>	=	meta
M	=	Millimolar
<i>m</i>	=	Multiplet (for NMR spectra)
<i>m/z</i>	=	Mass to charge ratio
M ⁺	=	Molecular ion
MCF-7	=	Human breast cancer cell line
MCF-7/Luc	=	Transfected MCF-7
MeCN	=	Acetonitrile
MEM	=	Minimal essential medium ,Eagle
MeOH	=	Methanol
MeOH- <i>d</i> ₄	=	Deuterated methanol
mg	=	Milligram
MHz	=	Megahertz

MIC	=	Minimum Inhibition Concentration
min	=	Minute
mL	=	Milliliter
MS	=	Mass Spectrometry
<i>Mult.</i>	=	Multiplicity
MW	=	Molecular weight
ND	=	Not determine
nm	=	Nanometer
nM	=	Nanomolar
NMR	=	Nuclear Magnetic Resonance
No.	=	Number
NOESY	=	Nuclear Overhauser Effect Spectroscopy
<i>o</i>	=	Ortho
ODS	=	Octadecylsilane
<i>p</i>	=	Para
PBS	=	Phosphate-buffered saline
PG	=	Prostaglandin
pM	=	Picomolar
ppm	=	Part per million
PR(-)	=	Phenol red negative
PR(+)	=	Phenol red positive
Rha	=	Rhamnose
RPMI	=	Rosewell Park Memorial Institute
s	=	Singlet (for NMR spectra)
spp.	=	Species
STS	=	Stilbene synthase
t	=	Triplet (for NMR spectra)
T47D	=	Human breast cancer cell line
T47D/Luc	=	Transfected T47D
t-DCTN	=	Trans-dehydrocrotonin
TLC	=	Thin Layer Chromatography
TMS	=	Tetramethylsilane
<i>t_R</i>	=	Retention time

UIGT	=	UDPG-isoflavonoid glucosyl transferase
UV	=	Ultraviolet
UV-VIS	=	Ultraviolet and Visible Spectrophotometry
VR	=	Vestitone reductase