


สารออกฤทธิ์ทางชีวภาพจากเห็ด *Coscinium fenestratum* (Gaertn.) Colebr



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จุฬาลงกรณ์มหาวิทยาลัย

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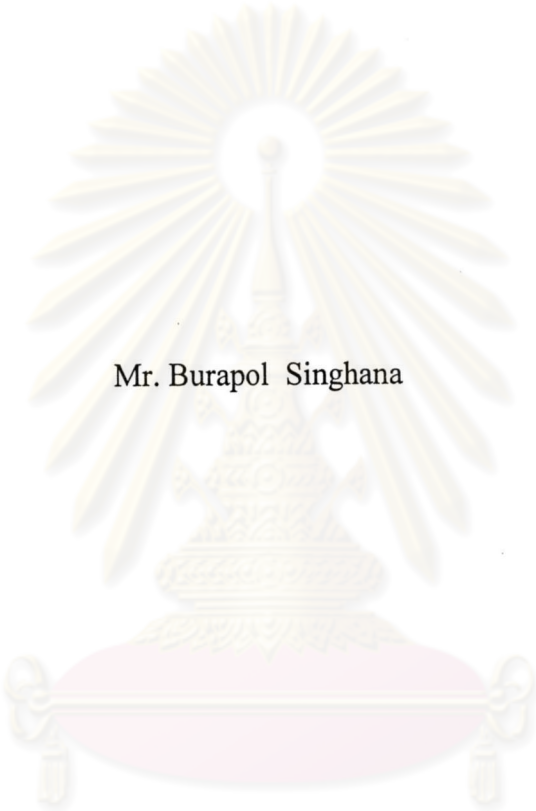
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BIOLOGICAL ACTIVE COMPOUNDS FROM *Coscinium fenestratum* (Gaertn.) Colebr



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งานวิจัยนี้เป็นการรายงานครั้งแรกเกี่ยวกับการค้นหาสารออกฤทธิ์ทางชีวภาพจากแห้ว *Cosciniium femestratum* (Gaertn.) Colebr วงศ์ Menispermaceae พบว่าสิ่งสกัดเอทานอลแสดงฤทธิ์ที่ดีมากต่อการต้านเซลล์มะเร็ง (KB, BC-1 และ NCI- H187) และแสดงฤทธิ์ปานกลางต่อการต้านเชื้อรา *Candida albicans* นอกจากนี้สิ่งสกัดเอทานอลยังแสดงฤทธิ์ต้านมาลาเรียและแสดงความเป็นพิษต่อโรสิตน้ำตาลและ *vero cells* ในการทดสอบฤทธิ์เบื้องต้น การแยกโดยใช้คอลัมน์โครมาโทกราฟีและพื้นฐานเทคนิคสเปกโทรสโกปีสามารถแยก ของผสมของสเตียรอยด์ (stigmastan-3,5,22-triene, stigmastan-3,5-diene, β -sitosterol, campesterol and stigmasterol), berberine, jatrorrhizine, เอสเทอร์ไฮดรอกซี, ของผสมซึ่งมี oleanolic acid, 8-oxotetrahydrothalifendine, 8-oxoberberine และ ของผสมของ steroidal glycoside (stigmasteryl-3-O- β -D-glucopyranoside และ β -sitosteryl-3-O- β -D-glucopyranoside), calonysterone และ ajugasterone D Calonysterone และ ajugasteron D ยังไม่มีรายงานมาก่อนในพืชชนิดนี้ ผลการทดสอบฤทธิ์ทางชีวภาพพบว่า berberine ซึ่งเป็นองค์ประกอบหลักแสดงฤทธิ์ต้านเซลล์มะเร็ง KB, BC-1 และ NCI- H187 ด้วยค่า IC_{50} 0.48, 0.95 และ 0.30 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ สารนี้แสดงฤทธิ์ต้านมาลาเรียด้วยค่า EC_{50} 0.11 ไมโครกรัมต่อมิลลิลิตร และแสดงฤทธิ์ความไม่เป็นพิษต่อ *vero cells* ด้วยค่า IC_{50} มากกว่า 50 ไมโครกรัมต่อมิลลิลิตร นอกจากนี้สารนี้แสดงฤทธิ์ต้านเชื้อรา *Candida albicans* ปานกลางด้วยค่า IC_{50} 13.0 ไมโครกรัมต่อมิลลิลิตร และแสดงฤทธิ์ต้านเชื้อวัณโรคด้วยค่า MIC 100 ไมโครกรัมต่อมิลลิลิตร Jatrorrhizine แสดงฤทธิ์ต้านเซลล์มะเร็ง NCI-H187 ด้วยค่า IC_{50} 0.08 ไมโครกรัมต่อมิลลิลิตร และแสดงฤทธิ์ต้านเชื้อวัณโรคด้วยค่า MIC 200 ไมโครกรัมต่อมิลลิลิตร นอกจากนี้ Jatrorrhizine ยังแสดงความเป็นพิษต่อ *vero cells* การศึกษา SAR ของ berberine และเกลือของมันพบว่า เกลือของ berberine บางตัว แสดงฤทธิ์ที่มีศักยภาพมากกว่าสารดั้งเดิม สำหรับการทดสอบฤทธิ์บางกรณี

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....
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BURAPOL SINGHANA: BIOLOGICAL ACTIVE COMPOUNDS FROM *COSCIINIUM*

FENESTRATUM (GAERTN.) COLEBR. THESIS ADVISOR: PROFESSOR UDOM

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This work is the first report concerning the investigation of biological active compounds from stems of *Coscinium fenestratum* (Gaertn.) Colebr. (Menispermaceae). It was found that ethanolic extract exhibited strong anticancer activity against cell lines (KB, BC-1 and NCI-H187) and displayed moderate activity against antifungal *Candida albicans*. In addition, it showed activity against antimalarial and exhibited low cytotoxicity against brine shrimp *Artemia salina* Linn. and *vero cells* in a preliminary screening test. The separation was performed by column chromatography and on the basis of spectroscopic techniques resulted in the isolation of a mixture of steroids (stigmastan-3,5,22-triene, stigmastan-3,5-diene, β -sitosterol, campesterol and stigmasterol), berberine, jatrorrhizine, a long chain ester, a mixture containing oleanolic acid, 8-oxotetrahydrothalifendine, 8-oxoberberine and a mixture of steroidal glycoside (stigmasteryl-3-O- β -D-glucopyranoside and β -sitosteryl-3-O- β -D-glucopyranoside), calonysterone and ajugasterone D. Calonysterone and ajugasterone D have not been reported as the constituents of this particular species. The result from bioactive test found that berberine, a major constituent, presented impressive anticancer activity against KB, BC-1 and NCI-H187 with IC₅₀ values of 0.48, 0.95 and 0.30 μ g/mL, respectively. This compound showed antimalarial activity with EC₅₀ value of 0.11 μ g/mL and displayed non-toxic activity against *vero cells* with IC₅₀ value of more than 50 μ g/mL. In addition, this compound exhibited moderate antifungal activity against *Candida albicans* with IC₅₀ value 13.0 μ g/mL and exhibited activity against antituberculosis activity with MIC value of 100 μ g/mL. Jatrorrhizine showed strong anticancer activity against NCI-H187 with IC₅₀ values of 0.08 μ g/mL and exhibited antituberculosis activity with MIC value of 200 μ g/mL. In addition, it displayed non-toxicity against *vero cells*. SAR study of berberine and its salts revealed that some salts of berberine showed more potent activity than original compound for some biological assays.

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 Field of study.....CHEMISTRY..... Advisor's signature.....*U. Kolep*.....
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LIST OF ABBREVIATIONS

| | |
|---------------------------------|------------------------------------------------------------|
| CDCl ₃ | deuterated chloroform |
| CHCl ₃ | chloroform |
| CH ₂ Cl ₂ | dichloromethane, methylene chloride |
| CIGAR | constant time inverse-detected gradient accordion rescaled |
| cm ⁻¹ | unit of wavelength |
| COSY | correlated spectroscopy |
| <i>d</i> | doublet (NMR) |
| DEPT | distortionless enhancement by polarization transfer |
| DMSO- <i>d</i> ₆ | deuterated dimethylsulfoxide |
| DMSO | dimethylsulfoxide |
| EtOAc | ethyl acetate |
| EtOH | ethanol |
| FT | fourier transform |
| HMBC | heteronuclear multiple bond correlation |
| HMQC | heteronuclear multiple quantum correlation |
| IC ₅₀ | 50% inhibition concentration |
| IR | infrared |
| <i>J</i> | coupling constant |
| LC ₅₀ | 50% lethality concentration |
| <i>m</i> | multiplet (NMR) |
| MeOH | methanol |
| MIC | minimum inhibition concentration |
| m.p. | melting point |
| MS | mass spectrometry |
| MW | molecular weight |
| <i>m/z</i> | mass to charge ratio |
| NMR | nuclear magnetic resonance |

LIST OF ABBREVIATIONS (continued)

| | |
|------------------|---------------------------------------------|
| NOE | nuclear overhauser effect |
| ppm | part per million |
| pyridine- d_5 | deuterated pyridine |
| R_f | retardation factor |
| s | singlet (NMR) |
| t | triplet (NMR) |
| TLC | thin layer chromatography |
| w/w | weight by weight |
| δ | unit of chemical shift |
| $\mu\text{g/mL}$ | part per million (microgram per milliliter) |



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