

**EVALUATION OF THE ANTI-BREAST CANCER ACTIVITIES
OF FURANODIENE AND GERMACRONE ISOLATED FROM
CHINESE MEDICINE *RHIZOMA CURCUMAE***

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CURCUMAE**

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A thesis submitted in partial fulfillment of the
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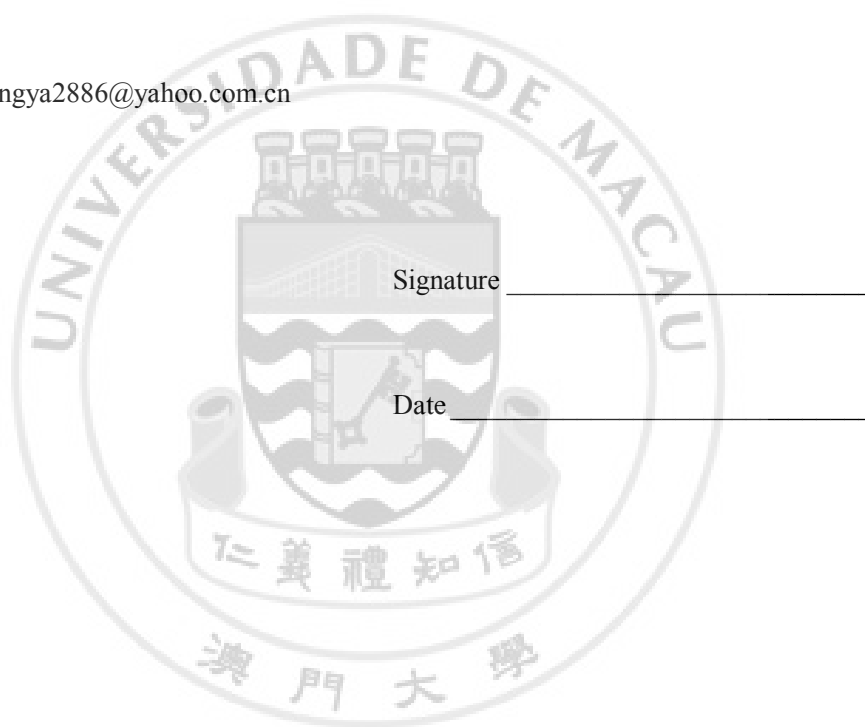
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碩士學位論文

中藥莪術中的呋喃二烯與吉馬酮的抗乳腺癌作用的研究

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by Zhangfeng Zhong

Thesis Supervisor: Prof. Yitao Wang

Chinese Medicinal Science

Abstract

Breast cancer occurs most frequently in women and it is the second leading cause of cancer deaths amongst women. *Rhizoma curcumae* (莪術 in Chinese) is widely prescribed in traditional Chinese medical formula for tumor therapy. Essential oils were the main bioactive constituents of *Ezhu*. Dozens of volatile oil components such as furanodiene, germacrone, curdione, curcumenol and curcumol *et al.* have been isolated and identified from *Ezhu*. *Ezhu* oil, furanodiene, germacrone could significantly inhibited the proliferation of MCF-7 and MDA-MB-231 cells in a time-dependent and dose-dependent manner, furthermore, furanodiene would enhance the growth inhibitory activity of tamoxifen (an anti-ER agent) in breast cancer cells, and curdione and curcumenol showed a little suppression effect on both two cells, but curcumol couldn't affect the cells proliferation. Furanodiene and germacrone also dramatically increased the LDH release in both cells. JC-1 fluorescence ratio (Red/Green) was dramatically decreased after 24 h of furanodiene and germacrone incubation in both cells suggesting the decrease/loss of $\Delta\Psi_m$. Flow cytometry showed that furanodiene suppressed MCF-7 cells growth by cell cycle arrest at the G0/G1 phase and germacrone suppressed both cells growth by cell cycle arrest at the G0/G1 and G2/M phase. MDA-MB-231 cells metastasis was inhibited by 25 μ M furanodiene.

Furanodiene could inhibit the proliferation of human umbilical vein endothelial cells (HUVECs) in a dose-dependent fashion but failed to inhibit VEGF-induced proliferation at low dose. Meanwhile, compared with VEGF induced control, the number of invaded cells and the migrated cells were significantly decreased in furanodiene treatment groups. Furthermore, furanodiene could dramatically suppress tube formation. Western blot results showed that furanodiene and germacrone affected the protein expression of PARP, integrin α V and cyclin D1. Those results indicated that two compounds germacrone and furanodiene isolated from Chinese medicine *Rhizoma curcuma* produces anti-breast cancer activities through suppressing cell proliferation, metastasis and angiogenesis via caspases, integrin and cyclin D1 pathway.

Keywords: *Rhizoma Curcumae*; germacrone; furanodiene; breast cancer;

中藥莪術中的呋喃二烯與吉馬酮的抗乳腺癌作用的研究

鐘章鋒 王一濤

摘要

乳腺癌多見於女性，導致婦女癌症死亡的第二大原因。莪術在傳統中醫藥的處方中廣泛用於治療腫瘤。揮發油是莪術的主要活性成分。幾十個莪術油的揮發成分，如呋喃二烯，吉馬酮，莪術二酮，莪術烯醇及莪術醇等已被分離和鑒定。莪術油，呋喃二烯，吉馬酮可顯著抑制乳腺癌 MCF - 7 和 MDA - MB - 231 細胞的增殖，並呈時效和量效關係。此外，呋喃二烯個還能增強他莫昔芬（一種抗雌激素受體劑）對乳腺癌細胞生長的抑制活性。莪術二酮和莪術烯醇對兩種子乳腺癌細胞也具有一定的抑制作用，但莪術醇不能影響細胞的增殖。呋喃二烯和吉馬酮也極大地增加了兩種細胞 LDH 的釋放。經過 24 小時呋喃二烯和吉馬酮的孵育後，細胞的 JC- 1 螢光比（紅/綠）的顯著減少提示線粒體膜電位的降低。流式細胞儀檢測細胞顯示呋喃二烯抑制 MCF-7 細胞的生長通過阻滯細胞在 G0/G1 期，吉馬酮抑制兩種細胞的生長通過阻滯細胞在 G0/G1 和 G2/M 期。乳腺癌 MDA - MB - 231 細胞的轉移的被 25 μ M

的呋喃二烯明顯抑制了。呋喃二烯能抑制人臍靜脈內皮細胞的增殖並呈量效關係，但在低濃度時不能抑制血管內皮生長因數誘導的增殖。與此同時，與血管內皮生長因數誘導組比較時，呋喃二烯處理組中遷移和侵襲的細胞數均顯著下降。此外，呋喃二烯可顯著抑制血管的形成。Western blot 結果表明，呋喃二烯和吉馬酮顯著影響了 PARP，整合素 αV 和 cyclin D1 等蛋白的表達。結果表明，莪術通過 caspase，整合素和 cyclin D1 通路抑制細胞的增殖，細胞的轉移和新生血管的產生，從而產生綜合的抗乳腺癌活性。

關鍵字： 莪術；乳腺癌；吉馬酮；呋喃二烯

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List of Abbreviations

Akt	Anti-apoptotic kinase
ATCC	American Tissue Culture Collection
BSA	Bovine serum albumin
ERK	Extracellular Signal-regulated Kinase
ER	Estrogen Receptor
EtOH	ethanol
FBS	fetal bovine serum;
Fur	Furanodiene
Ger	Germacrone
HUVEC	Human umbilical vein endothelial cell
LDH	lactate dehydrogenase
MAPK	mitogen-activated protein kinase
MTT	3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide
JC-1	5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl- benzimidazolylcarbocyanine iodide
PI3K	poly (ADP-ribose) polymerase
PBS	phosphate-buffered saline
PFA	paraformaldehyde
PI	propidium iodide
PMSF	phenylmethanesulfonyl fluoride
PS	penicillin-streptomycin
PVDF	polyvinylidene fluoride
TNFR1	tumor necrosis factor receptor 1
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
VEGF	Vascular endothelial growth factor