

**Discovery of Neuroprotective Compound from
Alpinia Oxyphylla by Multivariate Analysis of Chemical and
Bioactivity Profiles**

by

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Master of Science

2013



Institute of Chinese Medical Sciences

University of Macau

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A thesis submitted in partial fulfillment of the requirements for the degree of
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Approved by _____
Supervisor

Date _____

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碩士學位論文

應用多維變量分析技術從益智仁中分離一種 具有神經保護作用的化合物

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Abstract

Discovery of Neuroprotective Compounds from *Alpinia Oxyphylla* by Multivariate Analysis of Chemical and Bioactivity Profiles

By Shuai YUAN

Thesis Supervisor: Prof. Simon Ming-Yuan Lee

Chinese Medicinal Science

Parkinson's disease (PD), a degenerative disorder of the central nervous system, is the second neurodegenerative disorders among the elderly. The rapid increase in the number of PD patients without effective drug treatment urgently demands discovery and development of more effective PD management. Medicinal plants historically have been an important source for drug discovery. However, drug discovery from medicinal plants also faces many challenges, particularly due to the presence of complex chemical constituents, usually without well defined active components. Multivariate data analysis shall offer an alternative powerful solution to address this problem. With multivariate methods, one can investigate the relationship between all variables in a single context and these relationships can be displayed in plots for quick and convenient analysis.

In present study, we identified the neuroprotective constituent in ethanolic extract of *Fructus Alpiniae oxyphyllae* (AOE) using a PLS-DA model to analyze the correlation between the chemical profile and *in vitro* neuroprotective activity of eight

sub-fractions (Fr. A-H), generated from one-step chromatographic fractionation of EA part (ethyl acetate partition) of the AOE. By this way, a novel neuroprotective ingredient called compound 1 has been found. Compound 1 was further successfully separated and purified from Fr. D. The neuroprotective activity of compound 1 was further confirmed in both *in vitro* and *in vivo* experimental models of PD. Pretreatment with compound 1 exhibited significant neuroprotective activity against 6-OHDA-induced PC12 cell death. Moreover, treatment with compound 1 markedly protected loss of tyrosine hydroxylase (TH)-positive neurons and inhibited the swimming behavior reduction in the MPTP-induced PD model of zebrafish. In summary, the present study identified a novel neuroprotective compound (compound 1) from Fructus *Alpiniae oxyphyllae* by a new approach based on multivariate data analysis of chemical profile and neuroprotective activity of chemical fractions. Our result also demonstrated that compound 1 exhibited neuroprotective effects in both PD models of PC 12 cells and zebrafish. It suggests that compound 1 could be a promising agent for future development on prevention and therapy of PD.

Key words

Neuroprotective compounds, multivariate data analysis, PLS-DA, Parkinson's disease, Fructus *Alpiniae oxyphyllae*, zebrafish, PC12

摘要

帕金森病是一種中樞神經退行性疾病，在老年人當中，是目前第二大的神經退行性疾病。近年來，其迅速蔓延的趨勢和並無有效防治藥物的現狀，使得尋找更有效的治療手段的需求越來越迫切。歷史上，藥用植物一直是發現新型治療藥物的中藥來源。同時，隨著醫學的發展，人們認識到中藥所具有的獨特功能契合了醫藥發展的新需求。但是，中藥的複雜組成，特別是其化學組成的複雜性，是其發展面臨的重要挑戰。在解決這個問題當中，多維變量分析方法以其歸一化的數學計算方法以及易於理解的圖形表現方式，顯示了它的實力。

本課題在6-OHDA誘導的PC 12細胞損傷模型上，測定了益智仁乙酸乙酯提取物（AOE），其進一步分離產物（EA，PE，EO）以及EA部份的8個組分（Fr. A-H）的神經保護作用。進而，我們根據Fr. A-H的淨生物活性分佈構建其生物活性與化學信息的偏最小二乘判別模型（PLS-DA），以確定8個組分當中可能的生物活性成份。通過這種方法，我們發現了一種新型的神經保護物質 compound 1，並在體外及體內的帕金森病實驗模型進行確認。在6-羥基多巴胺（6-OHDA）誘導的PC-12細胞體外模型上，預處理compound 1 12小時明顯抑制了6-OHDA對PC 12 細胞的殺傷作用。同時，在1-甲基-4-苯基-1,2,3,6四氫吡啶（MPTP）誘導的斑馬魚體內模型上，compound 1也可有效抑制MPTP對斑馬魚多巴胺能神經元的損傷，恢復MPTP導致的斑馬魚運動減少的狀況。

綜上，compound 1可能成為一個有潛力的多靶點抗帕金森藥物。

關鍵字：神經保護；多維變量分析；偏最小二乘判別模型；神經退行性病；帕金森症；PC12細胞；斑馬魚；

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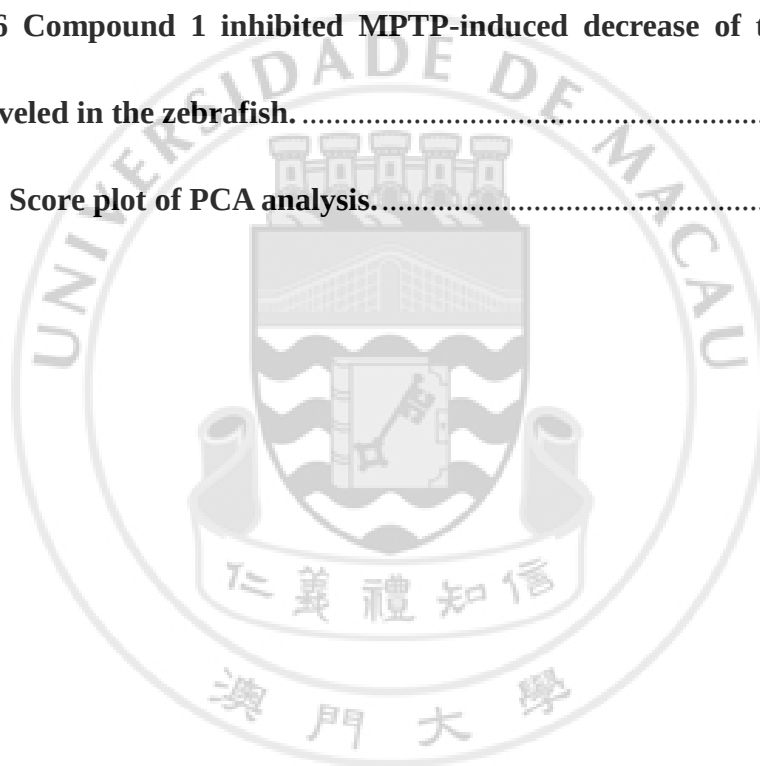
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ABBREVIATIONS

ND	Neurodegenerative diseases
AD	Alzheimer's disease
PD	Parkinson's disease
ALS	Amyotrophic lateral sclerosis
CNS	Central nervous system
DA	Dopaminergic
AOE	Ethanolic extract of Fructus Alpiniae oxyphyllae
PC 12	Rat pheochromocytoma cells
FBS	Fetal bovine serum
F-12K	Kaighn's modification of Ham's F12 medium
MTT	3-[4, 5-dimethyl- thiazol-2-yl]-2, 5-diphenyl tetrazolium bromide
DMSO	Dimethyl sulfoxide
PBS	Phosphate-buffered saline
6-OHDA	6-hydroxydopamine
BBB	Blood brain barrier
dpf	Days post fertilization
MPTP	1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
MPP+	1-methyl-4-phenylpyridinium ion
ROS	Reactive oxygen species
DAT	Dopamine transporters
NO	Nitric oxide
IL-1 β	Interleukin 1 beta
TNF- α	Tumor necrosis factor alpha

LRRK	leucine-rich repeat kinase
TH	Tyrosine hydroxylase
TCMs	Traditional Chinese medicines
NP	Natural products
SIMCA	Soft independent modeling of class analogy
PCA	Principle component analysis
PLS-DA	Partial least square regression-discriminant analysis
GFP	Green fluorescent protein
EDTA	Ethylenediaminetetraacetic acid
TLC	Thin layer chromatography
LC-MS	Liquid chromatography-mass spectrometry
ACN	Acetonitrile
IDA	Information dependent data acquisition
EMS	Enhanced mass spectrometry
LIT	Linear ion trap

