Tracing the Anti-Cancer Mechanism of *Pleurotus osteratus* Fractionate *via* Integrated Strategy of Network Pharmacology and Experimental Studies

Thesis submitted in partial fulfilment for the Award of Degree

Doctor of Philosophy

By

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Symbols

˚C	Degree Celsius
nm	Nanometre
µm	Micrometre
mm	Millimetre
cm	Centimetre
µL	Microliter
mL	Milliliter
g	Gram
µg	Microgram
kg	Kilogram
mM	Millimolar
M	Molar
%	Percentage
min	Minutes
sec	Seconds
v/v	Volume per volume
w/w	Weight per weight
vs	Versus
±	Plus minus
<	Less than
λmax	Wavelength at maximum absorbance
a.u.	Absorbance unit
Rf	Retention factor
Rt	Retention time
m/z	Mass-to-charge ratio

Abbreviation

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ACR	Acremoauxin A
AFPO1	Aqueous of DCM: Et crude extract of *Pleurotus osteratus*
ANOVA	Analysis of Variance
API	Apigenin
AST	Asterosterol
AVO1	Avocadyne 1-acetate
AVO4	Avocadyne 4-acetate
BET	Betulin
BFPO1	Butanol of DCM: Et crude extract of *Pleurotus osteratus*
BSA	Bovine serum albumin
CAR	Carbendazim
CEP	Cephaeline
CGA	Chlorogenic acid
CHOL	Cholest-5-ene
COUA	p-coumaric acid
DCM:Et	1:1 v/v dichloromethane: ethanol
DMEM	Dulbecco's Modified Eagle Medium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EFPO1	Ethyl acetate of DCM: Et crude extract of *Pleurotus osteratus*
ERE	(3b,6b,8a,12a)-8,12-epoxy-7(11)-eremophilene-6,8,12-trimethoxy-3-ol
ERG	Ergosterol
FBS	Fetal bovine serum
GC-HS	Headspace gas chromatography
GEPIA 2	Gene Expression Profiling Interactive Analysis 2

GO	Gene Ontology
HCA	Hierarchical Cluster Analysis
HFPO1	Hexane of DCM: Et crude extract of *Pleurotus osteratus*
HPTLC	High-performance thin-layer chromatography
IC_{50}	Half maximal inhibitory concentration
KAM	Kaempferol
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAB	(13R,14R)-7-Labdene-13,14,15-triol
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
LD_{50}	Median lethal dose
LIN	Linoleoyl ethanolamide
LOD	Limit of detection
LOQ	Limit of quantification
LUT	Luteolin
MOM	Momordol
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
MYX	Myxalamid B
NA	Naringenin
OBS	Beta-obscurine
OPH	Ophiobolin F
PA	Protocatechuic acid
PAN	D- pantothenic acid
PCA	Principal component analysis
PD1	DCM: Et crude extract of *Pleurotus roseus*
PD2	Hydroalcoholic crude extract of *Pleurotus roseus*

PE1	DCM: Et crude extract of *Pleurotus eryngii*
PE2	Hydroalcoholic crude extract of *Pleurotus eryngii*
PF1	DCM: Et crude extract of *Pleurotus florida*
PF2	Hydroalcoholic crude extract of *Pleurotus florida*
PLS	Partial least square
p.o.	Per oral
PO1	DCM: Et crude extract of *Pleurotus osteratus*
PO2	Hydroalcoholic crude extract of *Pleurotus osteratus*
POL	Polyporusterone G
PPI	Protein-protein interaction
PS1	DCM: Et crude extract of *Pleurotus sajor caju*
PS2	Hydroalcoholic crude extract of *Pleurotus sajor caju*
RHOD	Dihydroanhydorhodovibrine
RPM	Rotation per minute
s.c.	Subcutaneous
SD	Standard deviation
SE	Standard error
SEN	Senecionine
SOL	16,28-Secosolanidan-23-ol,3-amino-16,23-epoxy-, (3beta,5alpha,16alpha,22alpha,23beta,25beta)-
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
UALCAN	University of ALabama at Birmingham CANcer data analysis Portal

Preface

Pleurotus osteratus (*P. osteratus*), a species from the *Pleurotus* genus, has been intensively studied for its anti-cancer activity, attributed to higher molecular weight myco-metabolites. Conversely, little is known about the anti-cancer bioactivities associated with lower molecular weight myco-metabolites, to list a few ergosterols, and polyphenolic compounds claimed as lower molecular weight biomarkers responsible for anti-cancer activities of *P. osteratus*. Besides this, the comprehensive myco-metabolite profiling, the correlation between myco-metabolites and their bioactivities, and underlying mechanisms are still illusive.

The presented research work was designed to explore differential bioactivity-based screening of preferential extracted higher molecular weight, and lower molecular weight myco-metabolites, a detailed myco-metabolite profiling, and correlating with bioactivity and tracing mechanistic pathway involved in the anti-cancer intervention of potential one.

The thesis is divided into six chapters and are as follows:

Chapter 1 deals with the exhaustive review on explored anti-cancer potential of *Pleurotus* mushroom till date, an overview on chemometeric and network pharmacology, and along with rationale and research objectives. **Chapter 2** explores the bioactivity-based screening of different species of *Pleurotus* mushroom with their myco-chemical profiling. **Chapter 3** describes chemometric-based analysis of myco-metabolite of bioactive enriched fraction of *Pleurotus osteratus* (screened species) and its correlation with in-vitro cytotoxic activity. **Chapter 4** presents tracing the anti-cancer mechanism of HFPO1 (potential fractionate of *P. osteratus*) by the integrative approach of network pharmacology and experimental studies.

Chapter 5 documents tracing the anti-cancer mechanism of EFPO1 (potential fractionate of *P. osteratus*) by the integrative approach of network pharmacology and experimental studies. **Chapter 6** represents the overall summary and conclusion of the research work.