CITY UNIVERSITY OF HONG KONG
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The Quantitative Study, Fingerprint Analysis and Biological Effect of *Rhizoma Smilacis Glabrae*
土茯苓活性成分定量分析，指紋圖譜及生物活性研究

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Rhizoma Smilacis Glabrae (RSG) is a commonly used Chinese herbal medicine. To give scientific base for its applications, it is necessary to establish the chemical and biological profile of RSG. Two instrumental techniques, capillary electrophoresis (CE) and High Performance Liquid Chromatography (HPLC), were used for quantitative study and fingerprint analysis of RSG. Antioxidation and anti-cancer effects of RSG based on its chemical profile were also studied.

A CE method was developed for the separation and quantitative determination of six markers, namely trans-resveratrol, astilbin, taxifolin, shikimic acid, syringic acid and ferulic acid in RSG. The effects of borax and β-cyclodextrin (CD) concentration in electrophoretic buffer as well as its pH on the separation were systemically investigated. The optimal separation was carried out with running buffer of 20 mM borax containing 3 mM β-CD at pH 9.4. As the addition of CD in electrophoretic buffer significantly affected the electrophoretic mobilities of analytes, the complexation reactions of the six markers with different CDs (α, β, γ) were studied. Formation constant was calculated according to the electrophoretic mobilities change of analytes. The results showed that the size-fit relation between the host and guest was important for the complexation process. The developed quantitative method was successfully applied to determine the six components in 12 batches of RSG samples. Results revealed that astilbin was the most dominant component in RSG with content ranged from 11.5 to 27.6 mg g⁻¹, while ferulic acid, syringic acid and resveratrol could be absent. Furthermore, the quality of turtle jelly (Gui-ling-gao) was evaluated for the first time in terms of astilbin and taxifolin content.
by the CE method. Twenty one batches of samples with different brand were analyzed. Results showed that the content of astilbin and taxifolin in turtle jelly was distinctly different between brands, some even did not contain. Also, three commercial RSG concentrated extract products were analyzed and quality difference between brands was found.

For quality assurance and species authentication of RSG, its CE fingerprint was developed. To optimize the extraction condition, different extraction solvent and methods were compared. Methanol and sonication were recommended. Eighteen batches of RSG samples collected from various locations were investigated. RSG can be well distinguished from its two confusable species, Rhizoma Smilacis Chinae (RSC) and Rhizoma Heterosmilacis, by comparing their CE fingerprints.

HPLC fingerprint and quantitative analysis method was also developed for quality control and species distinguishing of RSG. Nine peaks were found in the chromatogram of RSG and all were identified by online electrospray ionization tandem mass spectrometry (ESI-MS/MS). These are 5-O-caffeoylshikimic acid, taxifolin, engeletin, isoengeletin, resveratrol, astilbin and its three stereoisomers. Among them, 6 constitutes were consistently found in 18 batches samples. The standard fingerprint of RSG was generated by mean simulation of the 18 tested samples. Based on the standard fingerprint, RSG can be easily distinguished from RSC and Rhizoma Heterosmilacis. Constitutes difference between RSG and RSC was further investigated by HPLC-ESI-MS/MS. Many constitutes, including shikimic acid, caffeoylshikimic acid, resveratrol, taxifolin, stereoisomers of astilbin and engeletin, were found in both species. However, ferulic acid and syringic acid were only found in RSG, while caffeoylquinic acid was only found in
The stability of RSG was investigated by monitoring the content of different constitutes at 55 °C for a period of 4 months. Result showed that the herb was stable during storage. The isomerization of astilbin during extraction was also investigated. Reflux (hot extraction) by solvent containing water would cause the isomerization of astilbin to its stereoisomers. Different extracts of RSG, including water extract, methanol extract and its ethyl acetate fraction, were prepared. The extracts were further analyzed by HPLC and CE, all extracts contained high content of dihydroflavonol glycosides such as astilbin, engeletin and their stereoisomers. Besides, phenolic acid caffeoylshikimic acid and shikimic acid were also contained. The dominant constitute in RSG, astilbin, was isolated and purified on a laboratory scale with purity of 95%. The method didn’t require repeated column chromatography or any special instruments. The product was characterized by element analysis; Ultraviolet-Visible spectrometry; mass spectrometry; IR spectrometry and nuclear magnetic resonance. Properties of astilbin were further investigated. Results showed that the water solubility of astilbin at 25 °C was about 250 μg/ml in acidic condition, and it was unstable in alkaline solution.

Different tests including radicals scavenging, reducing power and inhibition of linoleic acid peroxidation were employed to evaluate the antioxidant activities of astilbin and different extracts of RSG. All extracts showed concentration dependent antioxidant activity according to their contents of polyphenols. Polysaccharide did not show any antioxidant activity while purified astilbin showed the strongest antioxidant activity in comparison to any other extracts.

Methanol extract of RSG and astilbin showed cytotoxicity to HepG2, Hela and HL-60.
cells at relatively high concentration (all IC50>0.16 mg/ml). Morphological study with the method of acridine orange/ethidium bromide staining revealed that treating HepG2 cell with RSG would introduce the apoptosis with chromatin condensation and nuclear fragmentation. Cell cycle analysis showed that the pro-apoptotic effect of RSG was concentration and time-dependent and no phase arrest was noted. Although constitutes in RSC were quite similar with that of RSG, the cytotoxicity of RSC extracts to HepG2 cells was about twenty times stronger than that of RSG. Cell cycle analysis indicated that treating with methanol extract of RSC would cause G2/M arrest and then apoptosis of HepG2 cells. The pro-apoptotic effect was also concentration and time-dependent.
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ABBREVIATIONS

AA: Adjuvant-induced arthritis
ABTS: 2’-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt
AO/EB: Acridine orange/ethidium bromide
BHA: Butylated hydroxyanisole
BHT: Butylated hydroxytoluene
CD: Cyclodextrin
α-CD: α-Cyclodextrin
β-CD: β-Cyclodextrin
γ-CD: γ-Cyclodextrin
CE: Capillary electrophoresis
CGE: Capillary gel electrophoresis
CIEF: Capillary isoelectric focusing
CHM: Chinese herbal medicine
CZE: Capillary zone electrophoresis
DAD: Diode array detector
DMSO: Dimethyl sulfoxide
DNA: Deoxyribonucleic acid
DPPH: 1,1-diphenyl-2-picryl-hydrazil
ED: Electrochemical detector
EF: Ethyl acetate fraction
ESI-MS/MS: Electrospray ionization tandem mass spectrometry
EtOAc: Ethyl acetate
FDA: Food and Drug Administration
FTIR: Fourier transform infrared spectroscopy
FBS: Fetal bovine serum
GAP: Good Agricultural Policies
GC: Gas chromatography
HM: Herbal medicine
HPLC: High-performance liquid chromatography  
K: Formation constant  
LDH: lactate dehydrogenase  
MEKC: Micellar electrokinetic chromatography  
MRM: Multiple Reaction monitor  
MS: Mass spectrometry  
MTT: [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]  
NMR: Nuclear magnetic resonance  
NBT: Nitroblue tetrazolium  
NADH: Nicotinamide adenine dinucleotide reduced form  
OVI: Overlap index  
PA: Peak area  
PBS: Phosphate buffered saline  
PCA: Principal component analysis  
PMS: Phenazine methosulphate  
PPRC 2005: *Pharmacopoeia of the People's Republic of China 2005*  
PPRC 2010: *Pharmacopoeia of the People's Republic of China 2010*  
RP-HPLC: Reversed-phase High-performance liquid chromatography  
Rnase: Ribonuclease  
RPA: Relative peak areas  
RSC: *Rhizoma Smilacis Chinae*  
RSD: Relative standard deviations  
RSG: *Rhizoma Smilacis Glabrae*  
ROS: Reactive oxygen species  
SDS: Sodium dodecyl sulfate  
SES: Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine  
SF: Supernatant fraction  
SFDA: State Food and Drug Administration  
t<sub>R</sub>: Retention time  
t-BOOH: tert-butyl hydroperoxide
TCM: Traditional Chinese medicine
TLC: Thin-layer chromatography
TM: Traditional medicine
WE: Water extract
WHO: World health organization
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