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Anticancer effect of coniferyl alcohol on cholangiocarcinoma cell

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Introduction

Cholangiocarcinoma: combating a silent killer

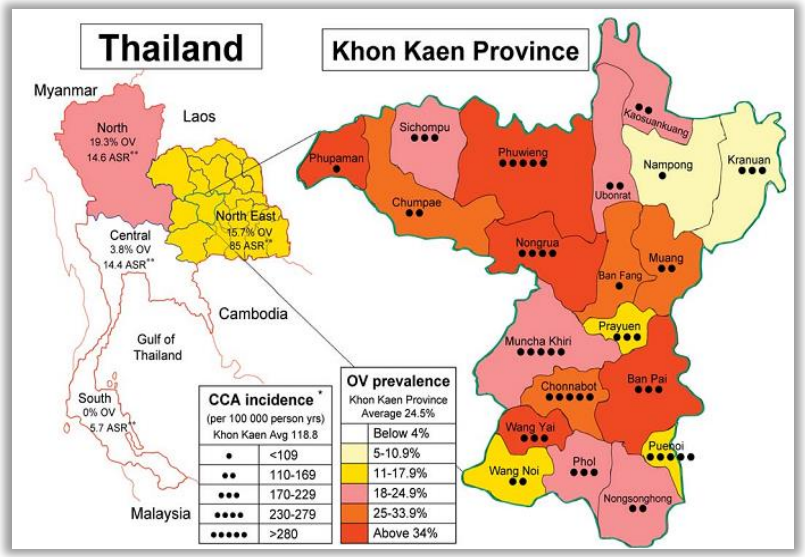


Figure 1 Epidemiology of CCA in Thailand

Figure 2 *Opisthorchis viverrini* metacercaria and their host (cyprinoid fish)

Treatment of cholangiocarcinoma

- ⇨ Operative therapy
- ⇨ Radiation
- ⇨ Chemotherapy
- ⇨ Chemoradiation treatment

Table 1 The toxicity of chemotherapy in advanced CCA patients

Adverse event	Gemcitabine based (N=21)		5FU based (N=84)	
	Grade 3, 4 N (%)	Grade 5 N (%)	Grade 3, 4 N (%)	Grade 5 N (%)
Leukopenia				
Anemia				
Thrombocytopenia	2 (9.52)	0	0	0
Neutropenia	3 (14.29)	0	1 (1.19)	0
Mucositis	0	0	1 (1.19)	0
Vomiting	0	0	0	0
Increased creatinine	1 (4.76)	0	0	0
Infection without Neutropenia	0	0	1 (1.19)	0
Infection with Neutropenia	1 (4.76)	1(4.76)	0	0
Biliary sepsis				
Hyponatremia				
Hypokalemia	1 (4.76)	0	0	0

*This table can not calculate P-value due to very small number in each parameter

Blood cell production

Electrolyte imbalance

The effective treatment with less toxicity of CCA is still needed

Introduction

Lignin as anticancer potential against cholangiocarcinoma cell

- Generally, Lignin found in cell wall of wood and bark.
- Lignins are cross-linked phenolic polymers.
- Three monolignol monomers are precursors including

  (1) paracoumaryl alcohol (PA)

  (2) sinapyl alcohol (SA)

  (3) coniferyl alcohol (CA) - 50-60%

- Lignin macromolecules are formed by the dehydrogenative polymerization of three monolignols with carbohydrates
- **50-60% of lignin's interunit linkage is β-O-4 unit**
- The pharmacological studies of lignin have been limited.

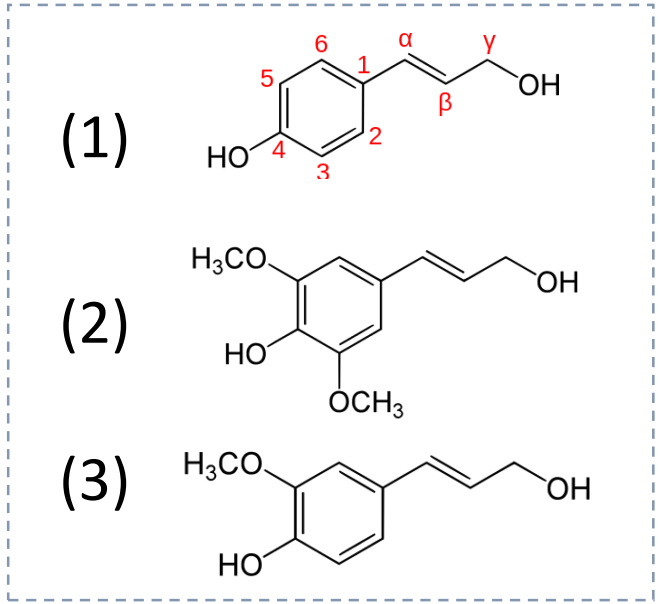


Figure 1 monolignol monomers

Lundquist, K. (1992).

Introduction

Lignin as anticancer potential against cholangiocarcinoma cell

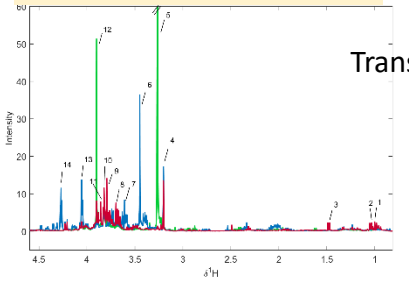
Sample preparation

C. halicacabum *G. celosioides* *S. dulcis*

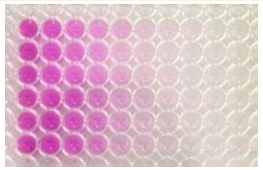
ECH EGC ESD

Plant extracts

NMR acquisition



Biological testing

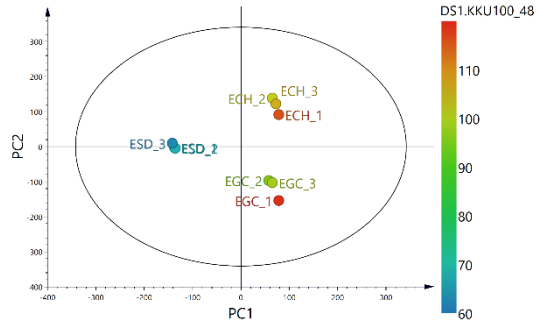


Transform variables

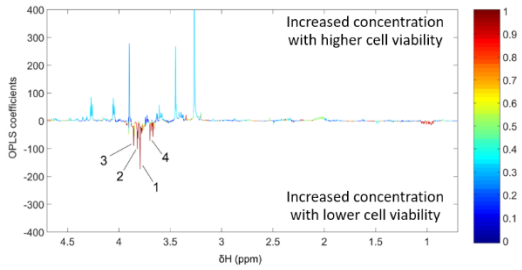
Generation of the datasets

Plant extracts	No.1	. . .	No. 43
Compound peak 1	$X_{1,1}$	. . .	$X_{43,1}$
.	.		.
.	.		.
Compound peak 634	$X_{1,634}$	. . .	$X_{43,634}$
%Cell viability	Y_1	. . .	Y_{43}

Multivariate statistical analysis

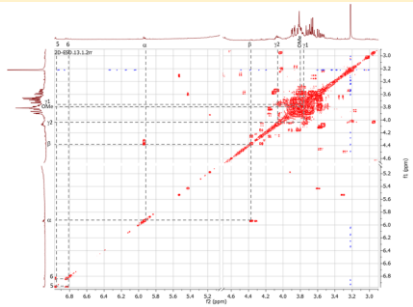


Identification of active metabolites



R^2X=0.83,
Q^2Y=0.99
P-value=0.02

2D-NMR for structure confirmation



Candidate compound

Lignin

Promraksa et al. *PLOS One;* 2019

Introduction

Metabolomics

- Metabolomics is the simultaneous (multiparallel) systematic identification and measurement of many cellular metabolites of a biological system at a specific point in time. It is a high-throughput analysis of metabolites.
- Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are often used for metabolome profiling as they produce rapid and reproducible results and samples can easily be prepared.
- NMR focuses on the metabolic profiling of **all of metabolites** (“fingerprint”) in a sample.

Untargeted metabolomics

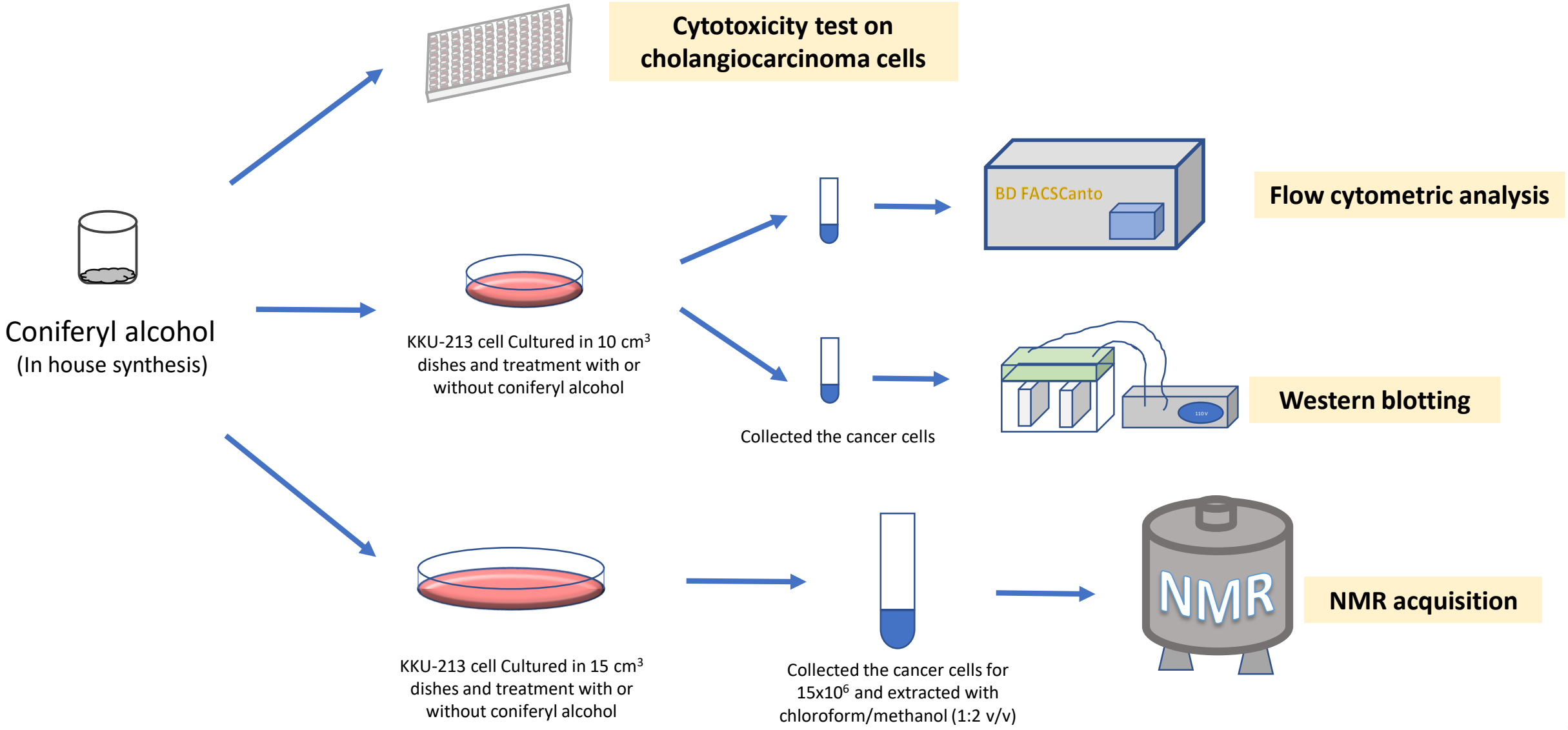
[1.]Nature [2.] Phytochem Rev (2008) 7:525–537

Research questions

- **Can coniferyl alcohol be inhibit the CCA cell viability?**

- **What are the key metabolites change after treatment with coniferyl alcohol that induced apoptosis in CCA cell line?**

Materials and Methods



Results

Coniferyl alcohol (CA) induced apoptosis

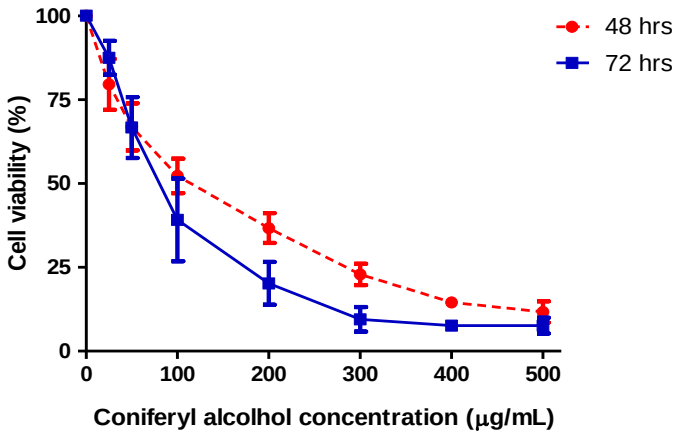


Figure 1 Viability of CA on KKU-213 for 48 and 72 hrs

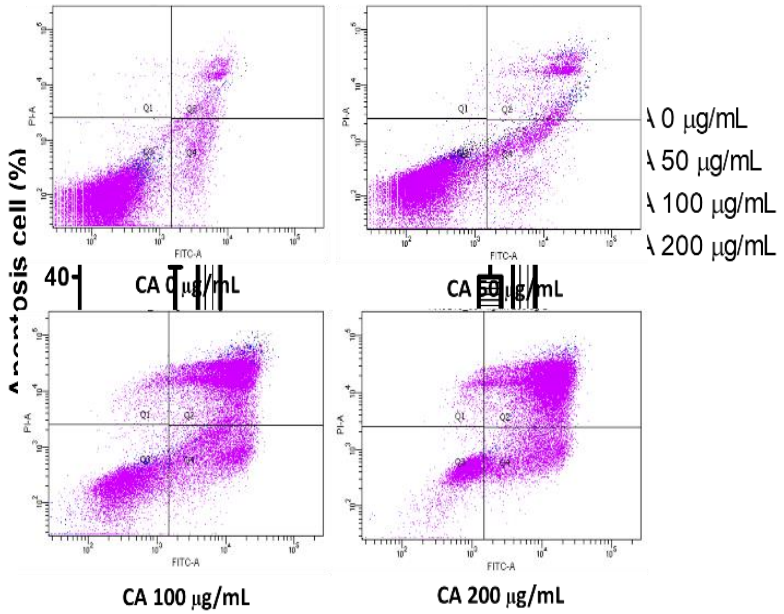


Figure 2 Flow cytometric analysis of apoptotic KKU-213 cells stained with PI and FITC-Annexin V after treatment with CA for 48 hrs

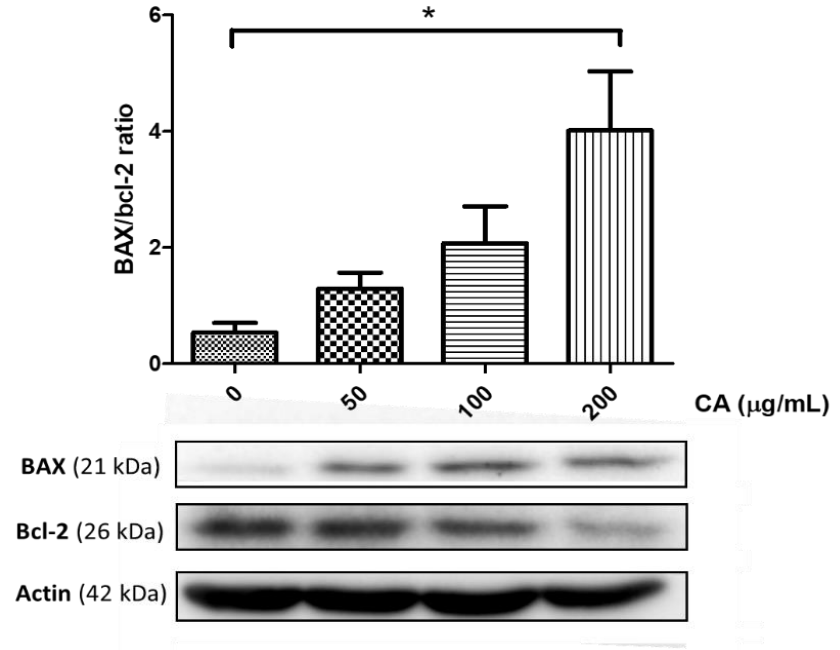


Figure 3 Effect of coniferyl alcohol on proapoptotic BAX and Bcl-2 protein in KKU-213 cells

CA can inhibit KKU-213 in dose dependent manner by inducing apoptosis

Results

Metabolic profiling of coniferyl alcohol induced KKU-213 cell apoptosis

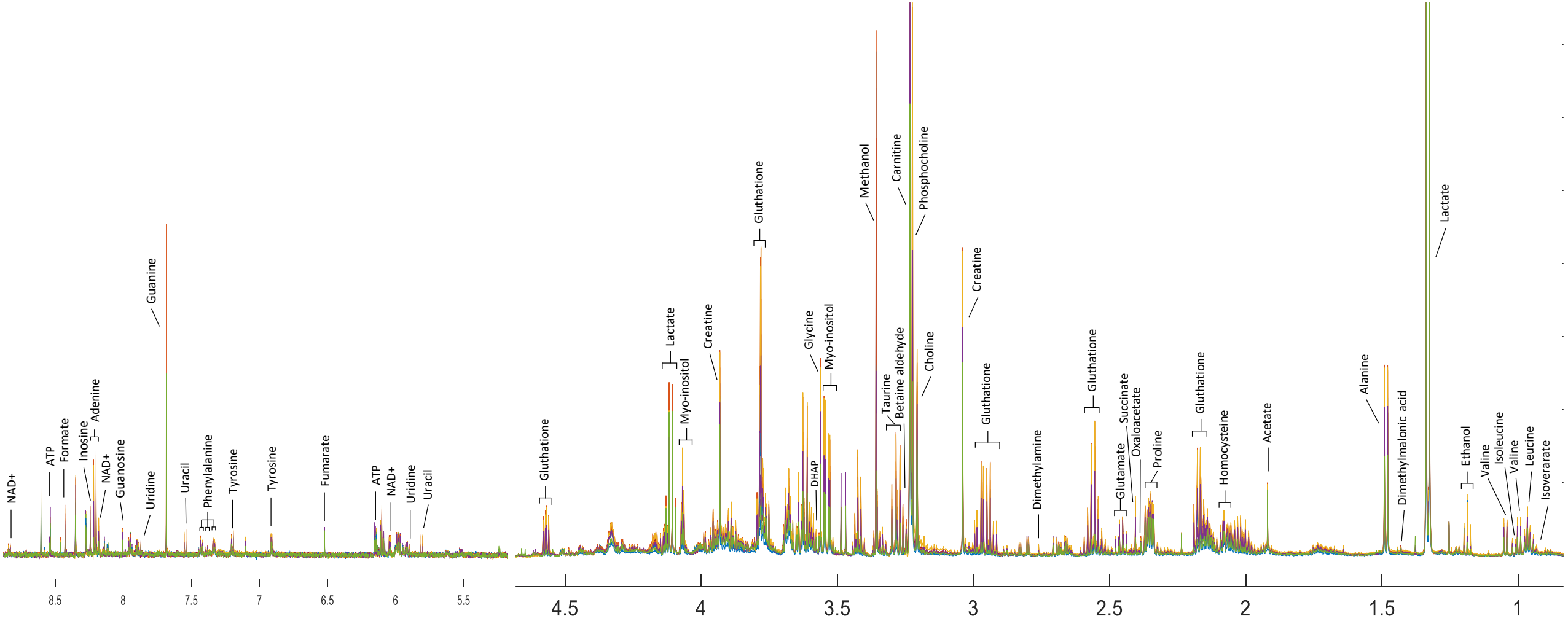


Figure 4 H^1 NMR spectra and metabolite identification of KKU-213 cell

Results

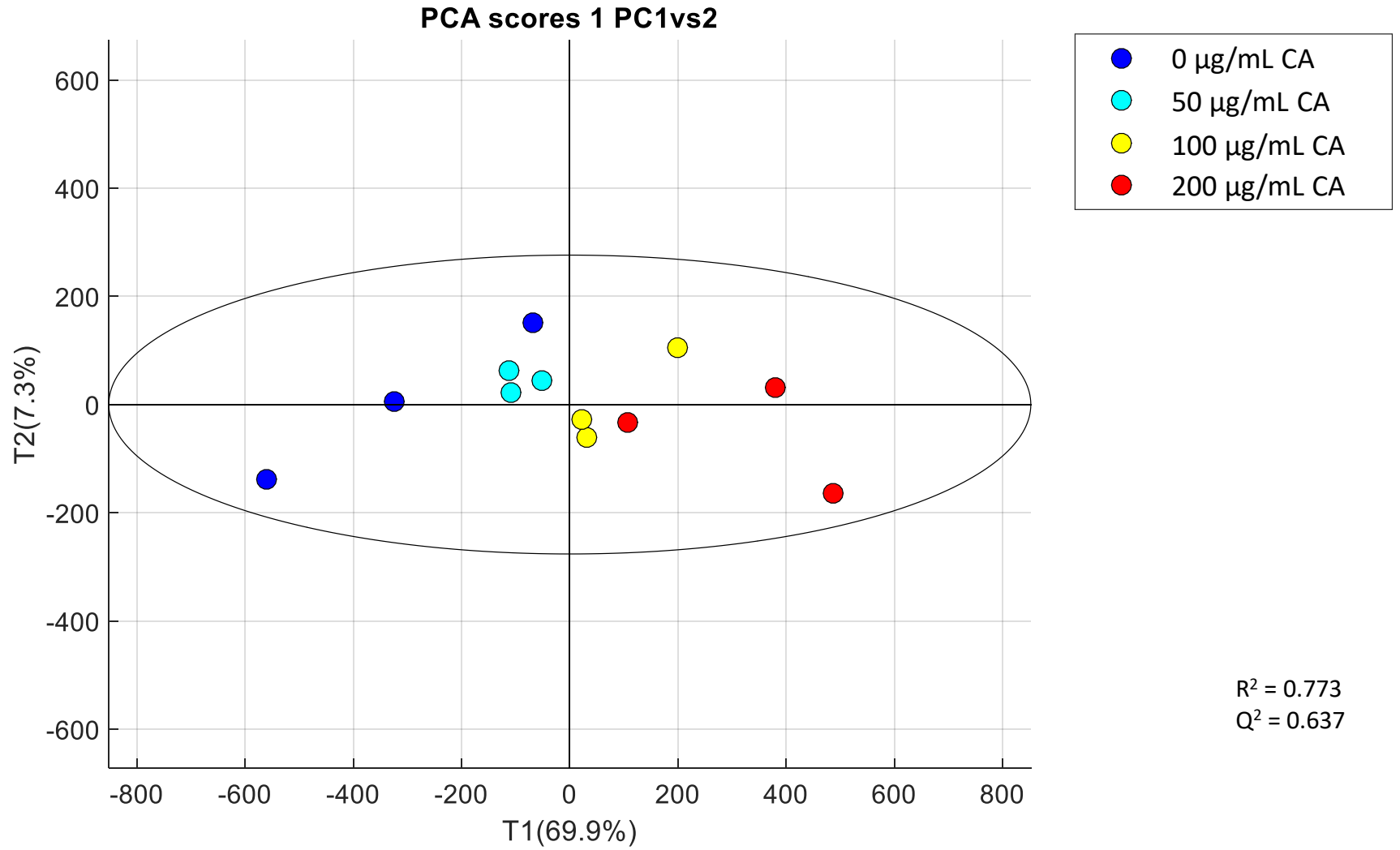


Figure 5 Principal component analysis of Intracellular metabolites of KKU-213 after treatment with or without CA

Results

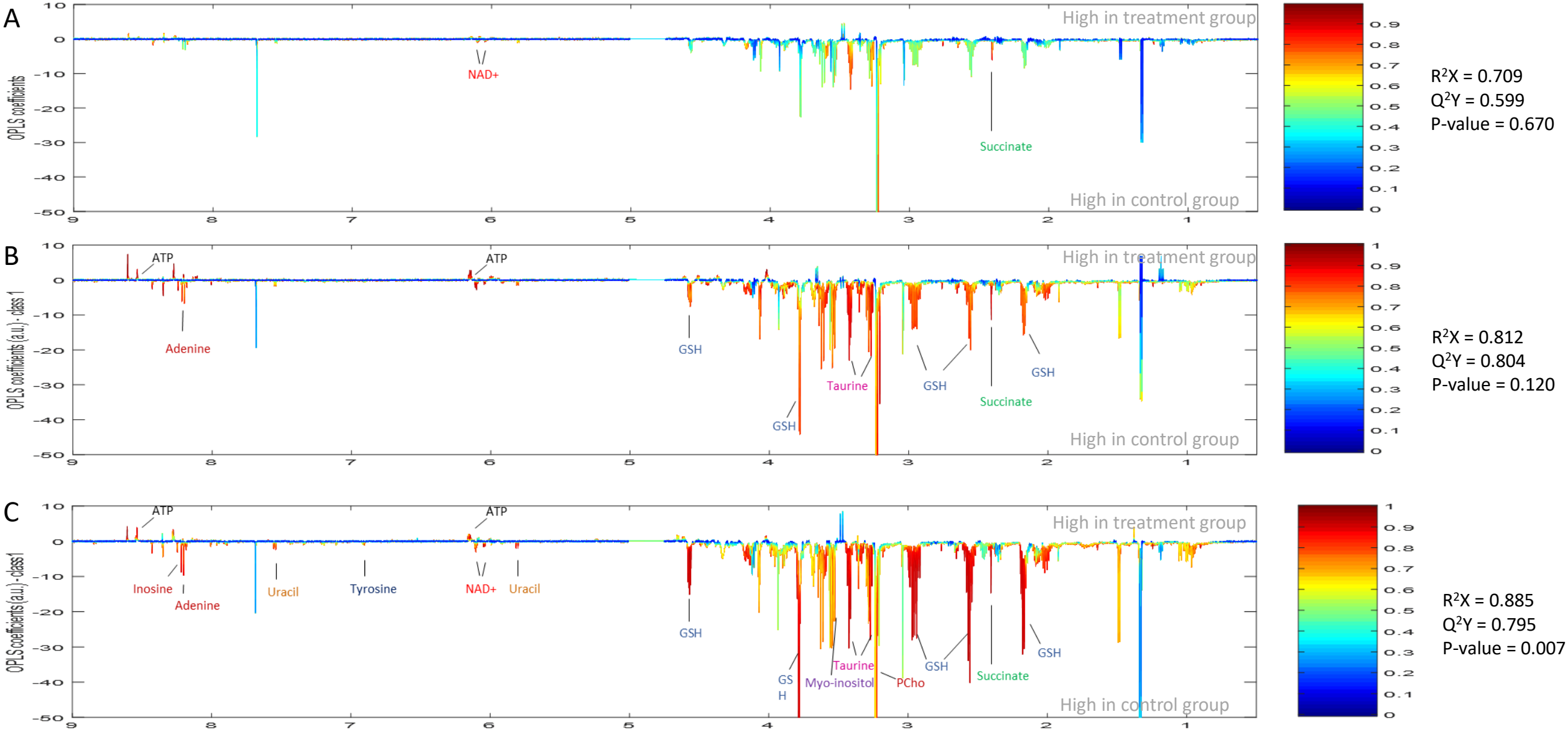


Figure 6 O-PLS corresponding coefficient loading plots displaying significant metabolites after treatment with or without CA

Results

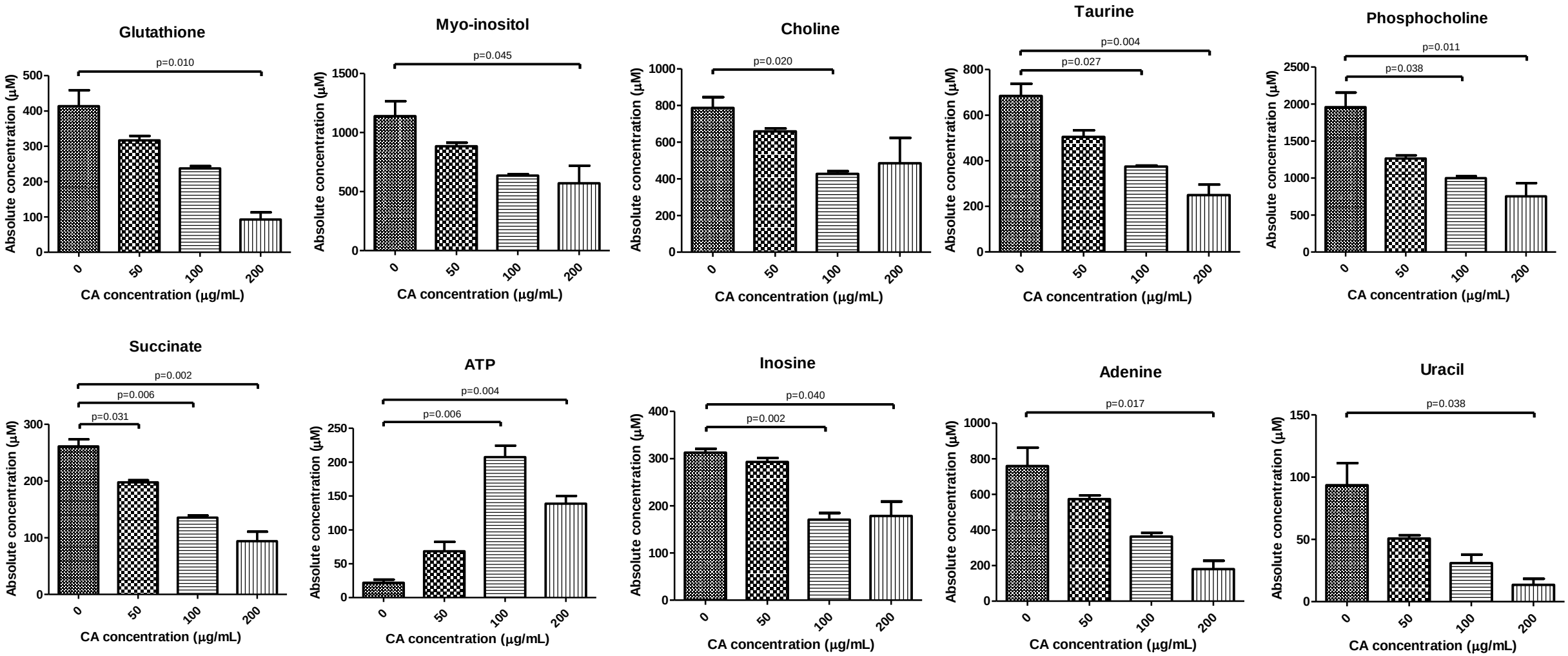


Figure 7 Comparison of significantly metabolites concentration

Discussion

- CA induced apoptosis in the cellular ratio of BAX/Bcl-2 that observed by western blot analysis and flow cytometry.

- The upregulation of BAX and downregulation of Bcl-2 protein results in the release of apoptogenic factors such as cytochrome c. In our results, ATP significantly increased which plays a critical role in early apoptosis as its interacts with apoptosis protease activating factor-1 (Apaf-1) before activation of caspase cascade pathway. At 200 ug/mL of CA treatment, KKU-213 cell partly underwent necrosis as observed in annexin V/PI staining *(Tsujimoto Y, 1997)*.

- Moreover, Glutathione depletion has important in cellular defense against reactive oxygen species (ROS) especially in apoptosis indicated that KKU-213 underwent the activation of the apoptotic signaling cascade *(Circu ML and Aw TY, 2012).*

- Previous study reported that choline-containing metabolites, taurine and glutathione were significantly decreased after treatment with doxorubicin which was in similarly to our results *(Opstad et al., 2009)*.

- Soares and coworker (2015) reported that inosine induces melanoma cell proliferation through phosphoinositide 3-kinase (PI3K) pathways. Therefore, inosine is associated with proliferation of melanoma cell proliferation. In our results, the lower of inosine content was present in CA treatment group compared to non-treatment group. It might be a potential marker for detection of CCA cell proliferation.

Reference

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- Tsujimoto Y. Apoptosis and necrosis: Intracellular ATP level as a determinant for cell death modes. *Cell Death Differ.* 1997.4; 429–434.
- Opstad KS, Bell BA, Griffiths JR, et al. Taurine: a potential marker of apoptosis in gliomas. *Br J Cancer*. 2009 Mar 10;100(5):789-94.

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