

Anti-diabetic activity of canophyllol from *Cratoxylum cochinchinense* (Lour.) Blume in type 2 diabetic mice by activation of AMP-activated kinase and regulation of PPAR γ

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S1. Screening Methodology Validation

There are several papers reported that IRAP-mOrange and GLUT4-eGFP could be applied to detect the GLUT4 translocation in L6 (Wang et al., 2009; Zhou et al., 2016; Huang et al., 2016) and 3T3-L1 cells (Bai et al., 2007; Jiang et al. 2008). In order to validate the feasibility of our IRAP translocation assay for discovering potential hypoglycemic agents, we have observed the effects when the GLUT4-eGFP or IRAP-marked L6 cells treated with insulin and berberine which are definitely pharmacodynamic GLUT4 agonists. L6 cells which stably express IRAP-mOrange and GLUT4-eGFP were cultured in α -MEM supplemented with 10% fetal bovine serum and 1% antibiotics (100 U/mL penicillin and 100 μ g/mL streptomycin) at 37 °C in 5% CO₂. L6 cells was seeded in 48 well plates, and incubated until 100% confluence and then starved in serum-free MEM- α for 2 h. Afterwards, L6 cells were treated with insulin (10 nM) and berberine (5 μ M). The cells were taken photos with a laser-scanning confocal microscope LSM 510 (Carl Zeiss, Jena, Germany) to supervise the IRAP-mOrange and GLUT4-eGFP translocation. And the images were captured with 555 nm excitation laser every 10 seconds in first 5 minutes and then every 5 minutes in later 30 minutes.

During the experiment, as time went on, we could observe the green and red fluorescence enhanced significantly after treating with insulin and berberine in L6 cells (Fig. 1). The results showed that GLUT4 and IRAP simultaneously translocated onto the plasma membrane in 30 min when adding the GLUT4 agonist. GLUT4 has mainly been recruited to the PM throughout to the GLUTs storage vesicles (GSV). Three main proteins stored in GSV are GLUT4, IRAP, and Sortilin (Shi et al., 2005). It was reported that IRAP and GLUT4 displayed a strong colocalization (Kumar et al., 2010; Rubin et al., 2009) in many researches. Thus, detecting the IRAP can indirectly reflect the situation of GLUT4. So our results could be explained that detecting the IRAP-mOrange fluorescence could indirectly reflect the GLUT4 translocation. As the

red fluorescence is more conspicuous than green fluorescence for observation, so we choose the IRAP-mOrange fluorescence assay for reflecting GLUT4 translocation.

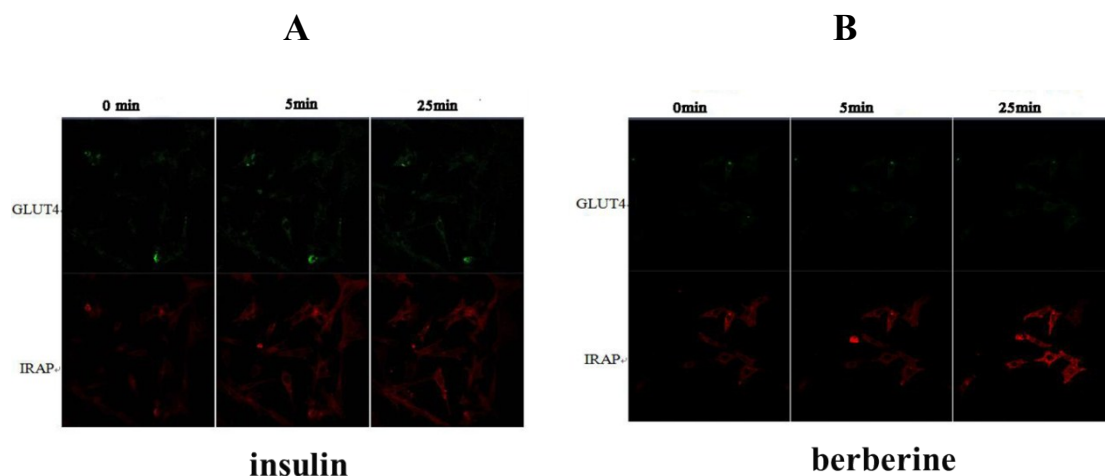


Figure. 1 L6 cells were infected with IRAP-mOrange and GLUT4-eGFP in order to detect externalized GLUT4 translocation by confocal microscopy. (A) Confocal images in L6 cells incubated in the absence (0 min) or presence of insulin for 5min, 30 minutes. (B) Confocal images in L6 cells incubated in the absence (0 min) or presence of berberine for 5 min, 30 minutes.

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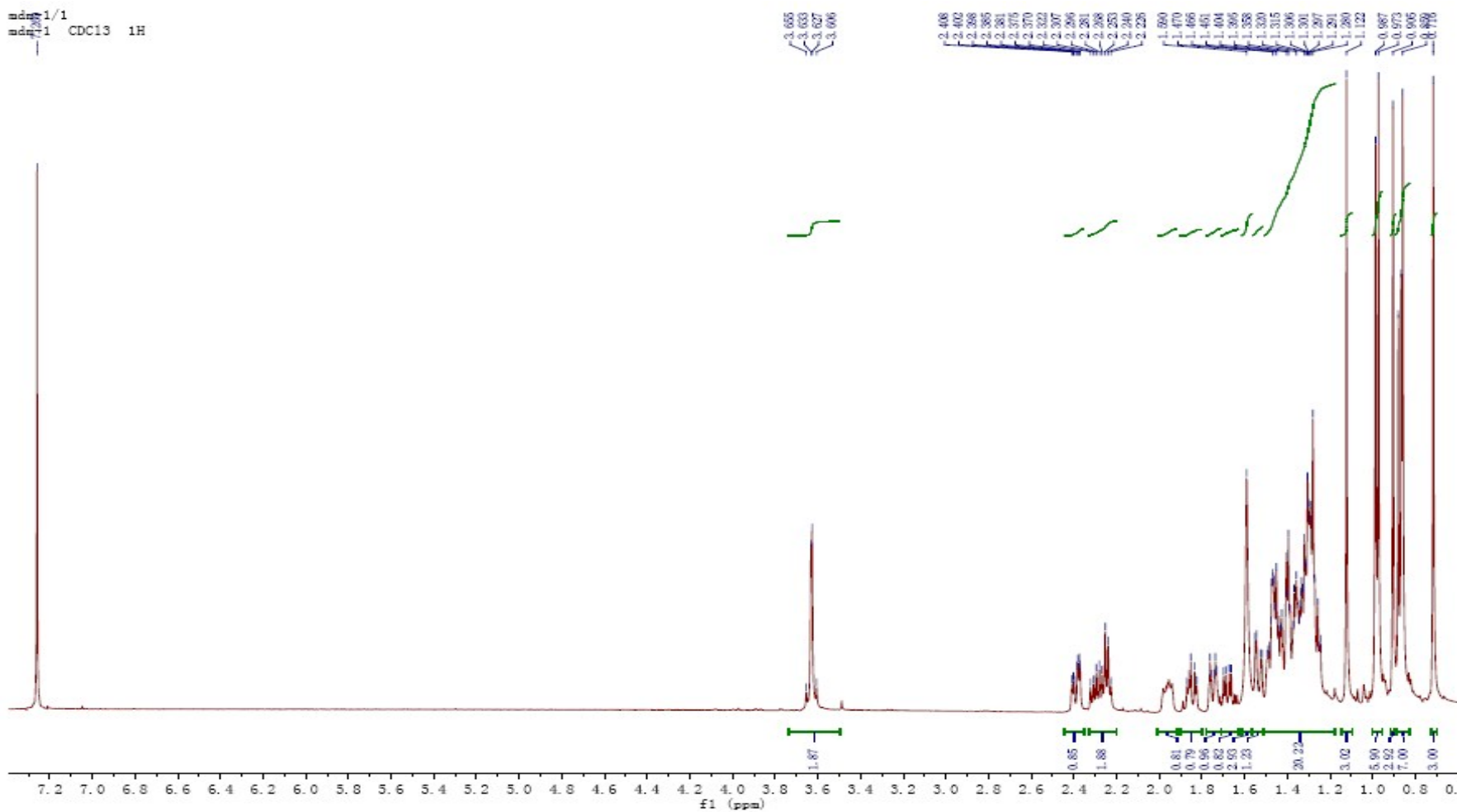
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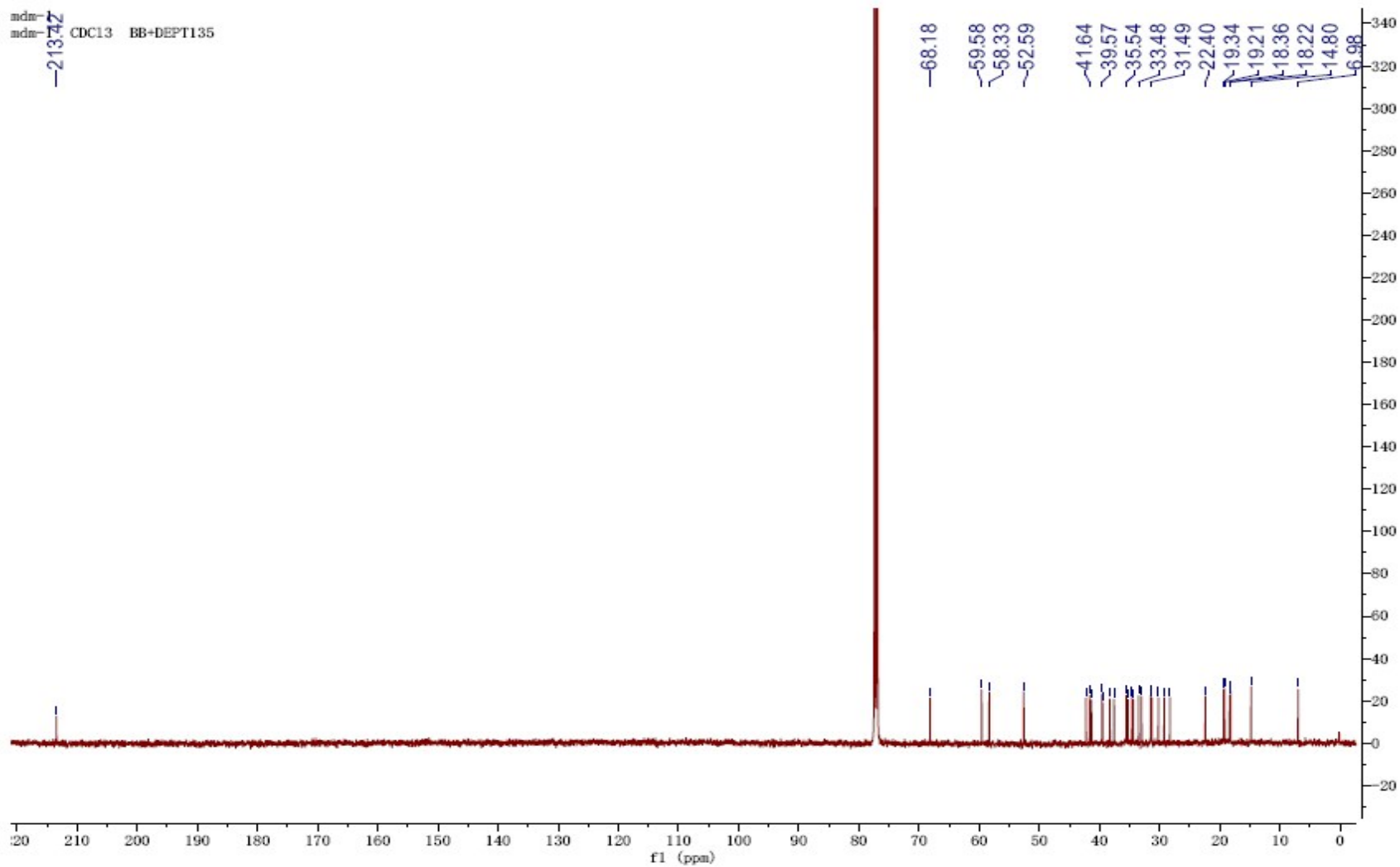
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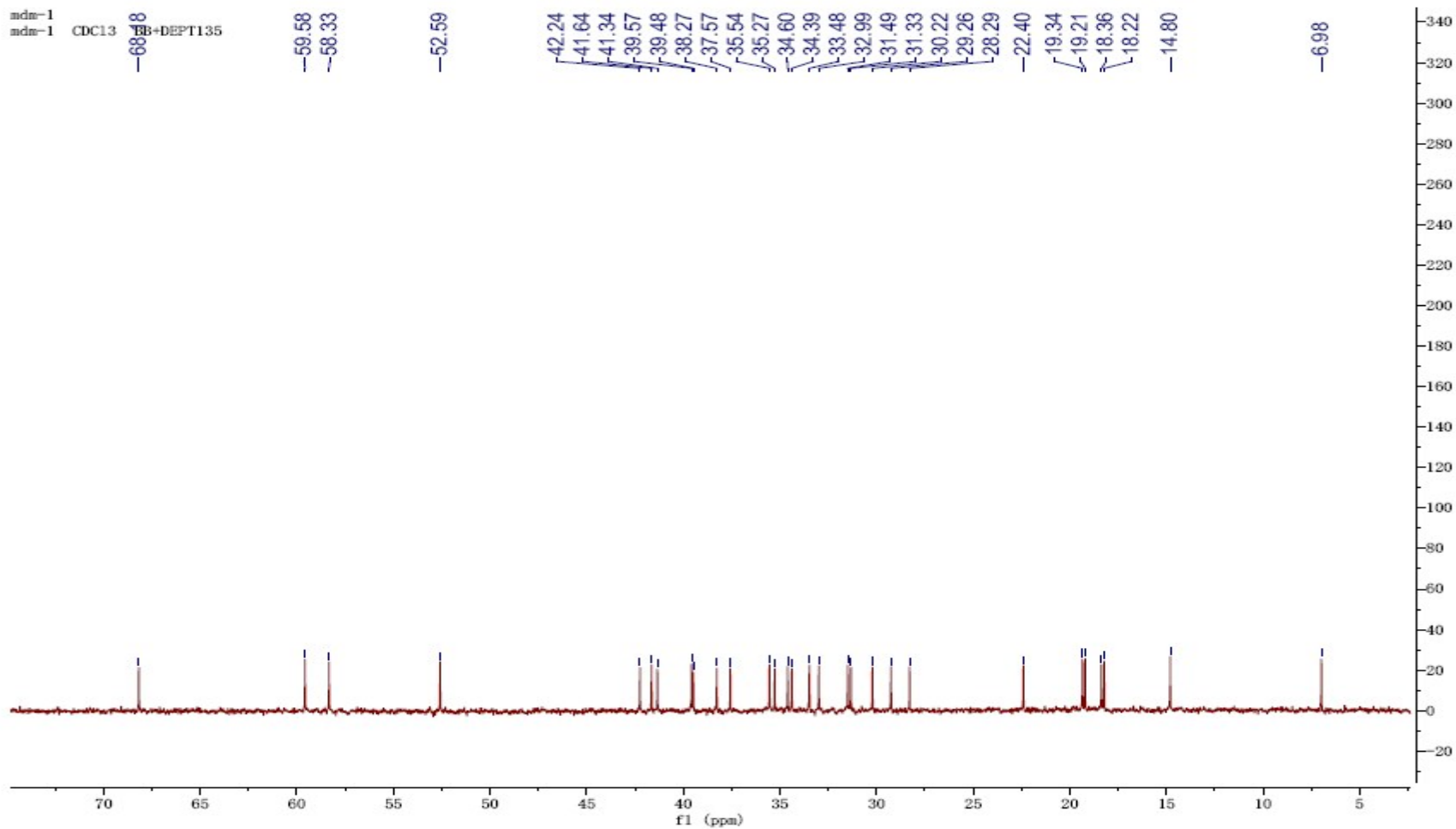
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S2. ¹H-NMR spectrum of canophyllol



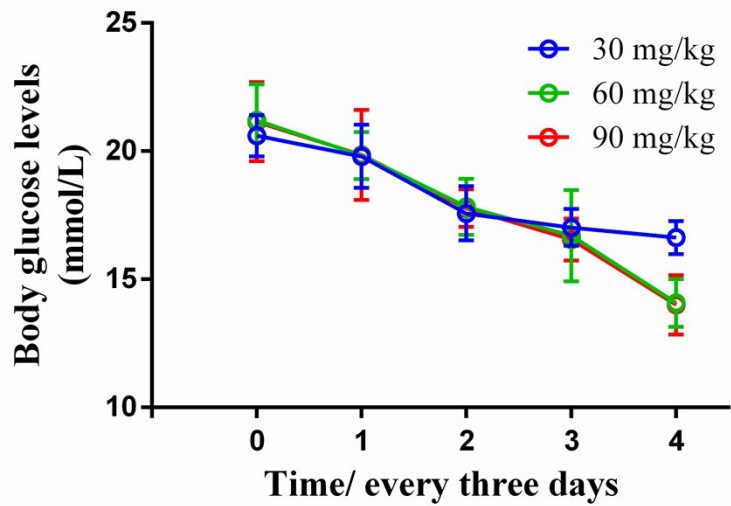
S3. ^{13}C -NMR spectrum of canophyllol



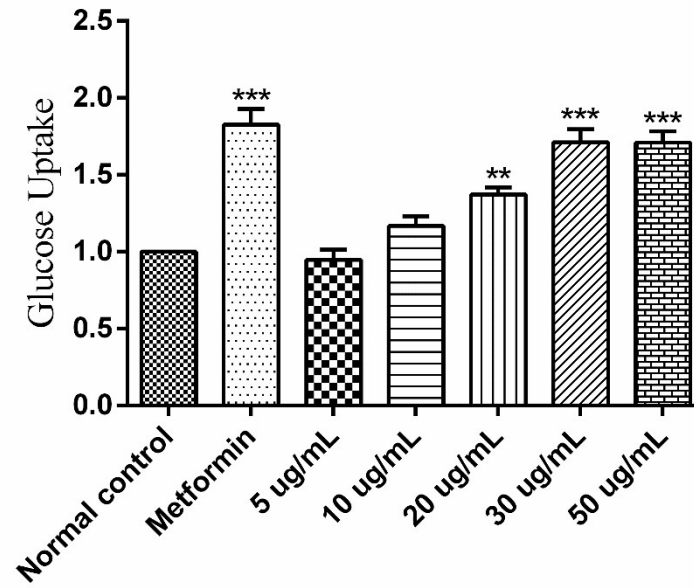
S4. Part enlarging of ^{13}C -NMR spectrum of canophyllol

<div style="text-align: center;">Informations</div> <div style="text-align: left;">Constituents</div>	Content
Protein	225 g/Kg
Fat	200 g/Kg
carbohydrate substances	450 g/kg
Cholesterol	12.5 g/Kg
Sodium cholate	5 g/Kg
Energy	4500 kcal/Kg

S5. Detailed information of High-fat diet



Pre-studies result for dosages of mice



Pre-studies result for glucose uptake in cells

S6.Results of pre-studies