



# 6th International Electronic Conference on Medicinal Chemistry

1-30 November 2020

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## Saponin from *Acacia concinna* (Wild.) DC. inhibits pancreatic lipase and enhance lipolysis in 3T3-L1 adipocyte

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**HOKKAIDO**  
UNIVERSITY

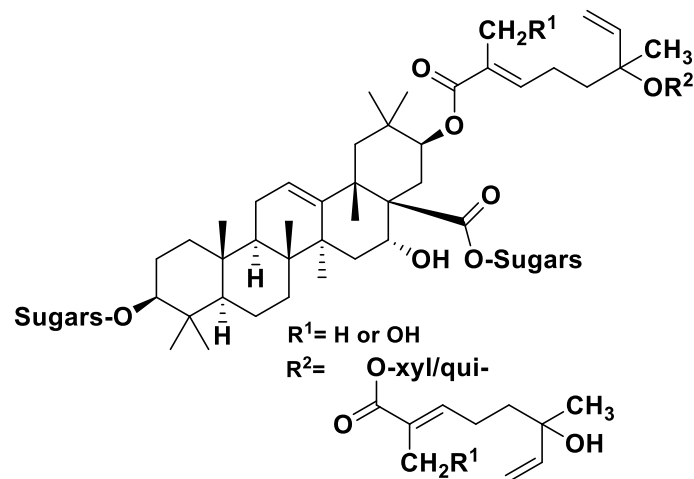
# Saponin from *Acacia concinna* (Wild.) DC. inhibits pancreatic lipase and enhance lipolysis in 3T3-L1 adipocyte

## Graphical Abstract



*Acacia concinna* pod

Anti-obesogenic  
bioactive principle



*A. concinna* Saponin

Pancreatic lipase inhibition:  $IC_{50} = 7.93 \mu\text{g/mL}$  extract  
3T3-L1 lipolysis enhancement:  $EC_{50} = 58 \mu\text{g/mL}$  extract



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## Abstract:

Overweight and obesity are turning into serious health problem. Natural products contained in medicinal plants are expected to be an effective source for the treatment of obesity. *Acacia concinnais* a medicinal plant, which was previously found to have high anti-obesogenic potential. However, the bioactive compound contained in the plant is undiscovered. In this study, we isolated the bioactive compound in the pod of *A. concinna* and evaluated its activity to inhibit pancreatic lipase and enhance lipolysis in 3T3-L1 adipocyte, two bio-activity related to anti-obesogenic potential.

Chromatographic purification of the extract of *A. concinna* pods guided by pancreatic lipase inhibitory activity assay gave mixture of saponins as the bioactive principle of *A. concinna* pods. Structural analysis by acidic or alkaline breakdown followed by chromatographic purification and spectrometric analysis gave acacic acid (triterpene), monoterpene with sugar (quinovose or xylose), and several sugars (glucose, arabinose, rhamnose) as components of the saponin showing resemblance to the reported structures.

Anti-obesogenic potentials of the *A. concinna* saponins were evaluated for lipase inhibition and enhancement of lipolysis in 3T3-L1 adipocyte. The results indicated that the saponin is an efficient lipase inhibitor and a lipolysis enhancer. In addition, the saponin also reduced the lipid accumulation in mature adipocytes. Thus, *A. concinna* saponin might be a good source for the treatment of obesity.

**Keywords:** 3T3-L1; anti-obesogenic; bioactive compounds; lipase; lipolysis



# Obesity treatment

## Drugs: treat obesity

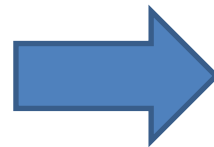
- Cetilistat
- Empatic
- Liraglutide
- Etc.



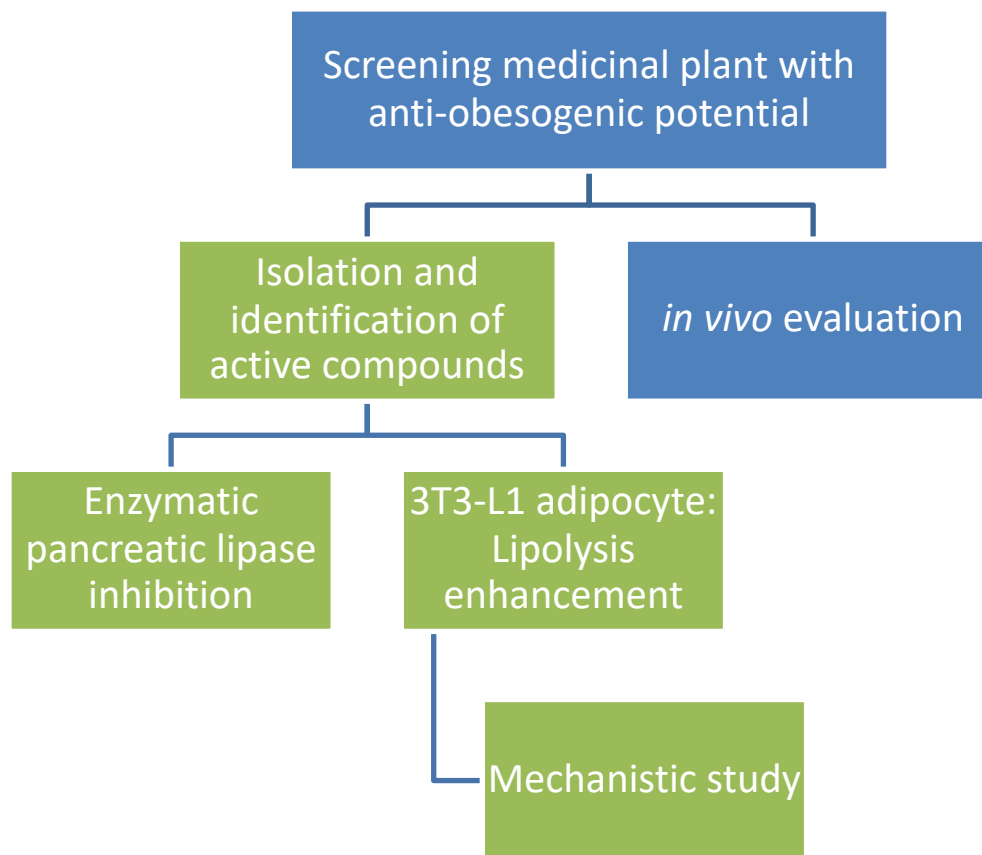
## Side effects:

- nausea
- constipation
- sleep disturbance
- Etc.

**Traditional remedy**  
less side effects



# Overall scheme of this project

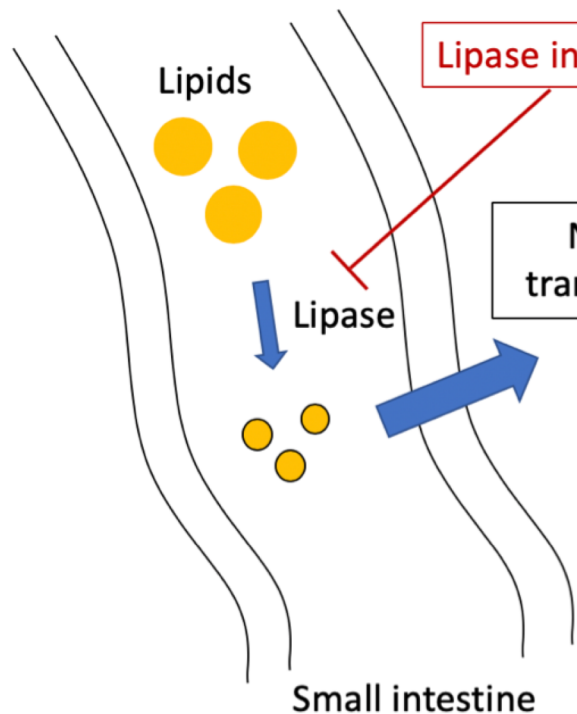


**Aim of this study: to find active compounds from the candidate plant selected by screening and evaluate its anti-obesogenic potential**

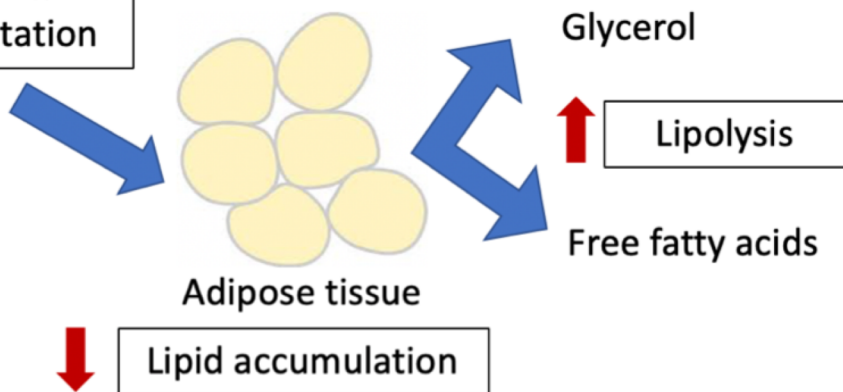


# Screening to find anti-obesogenic candidate plant

## I. Enzymatic assay: Pancreatic lipase inhibition



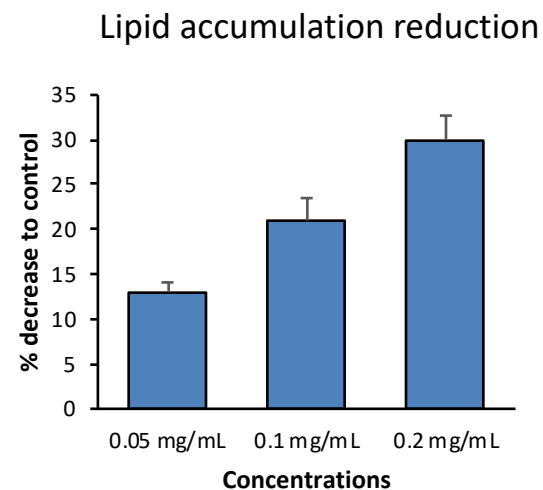
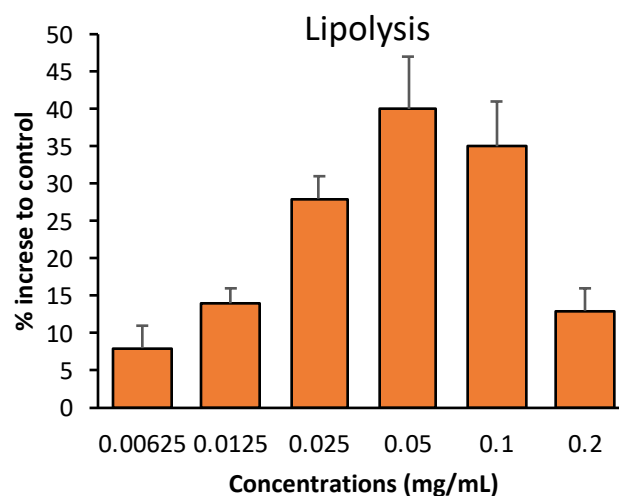
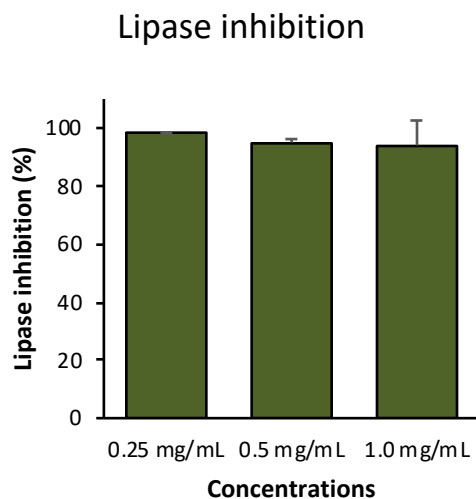
## II. Cell-base assay: Lipolysis enhancement



## III. Cell-base assay: Lipid accumulation reduction



# Anti-obesogenic activity of *Acacia concinna* extract



Ruangaram W, Kato E. *Pharmaceuticals*, 2020, 13(4): 56.

## Result

*Acacia concinna* was found as one of the **promising plant with anti-obesogenic potential**

## Objective

To find **active compounds** in pod of *Acacia concinna*



# ***Acacia concinna* (Wild.) DC.**

**Common name:** Soap pod

**Family:** Fabaceae

**Part of used:** pods

**Traditional uses:** Laxative effect, cough, antidandruff

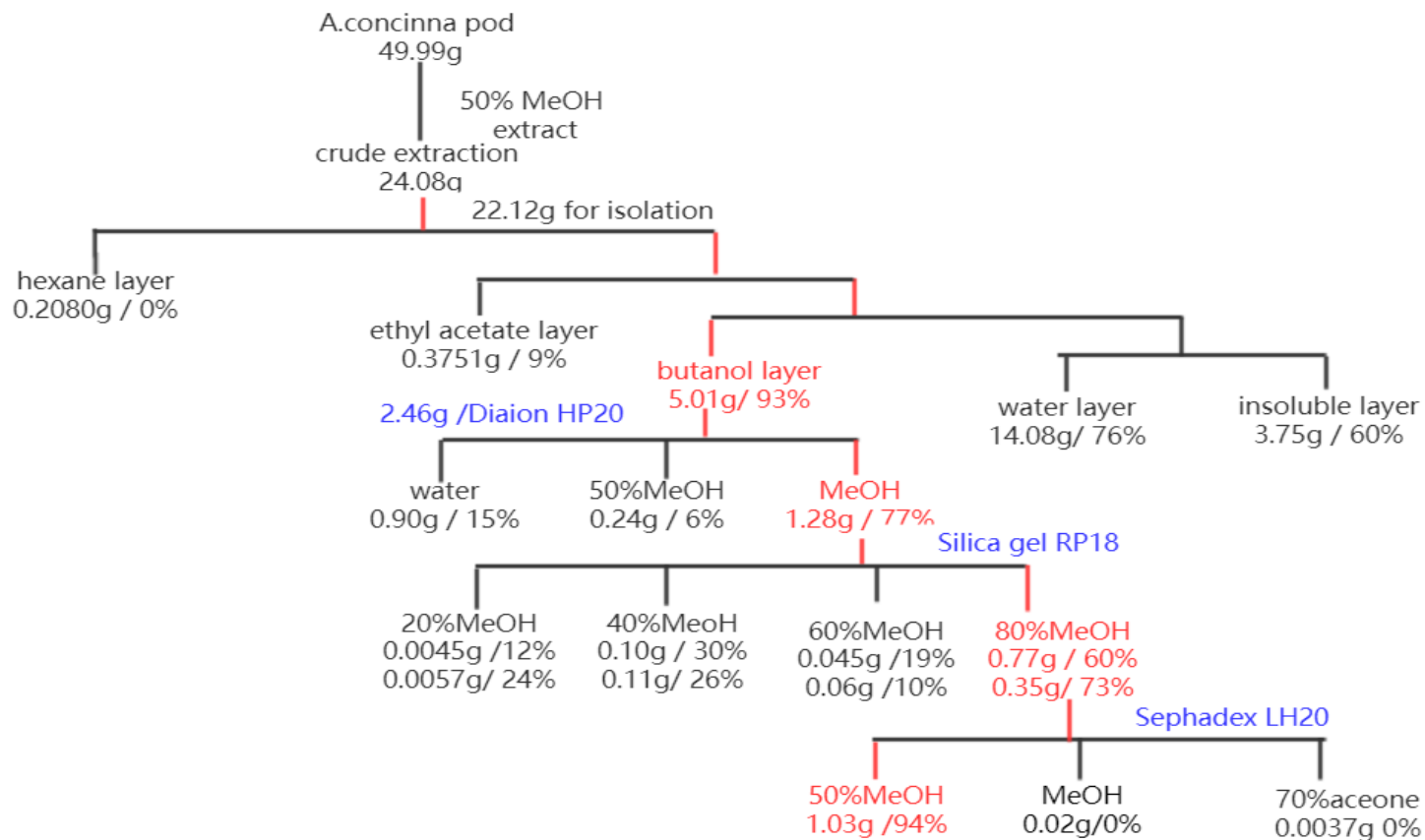
## **Research:**

- Antioxidant, anti-coagulant, anti-platelet, anti-thrombotic, anti-dermatophytic and immune adjuvant activities
- consisted of several alkaloids, flavonoids, saponin and tannin





# Fractionation



**Active fraction**

Yield/ Lipase inhibitory activity



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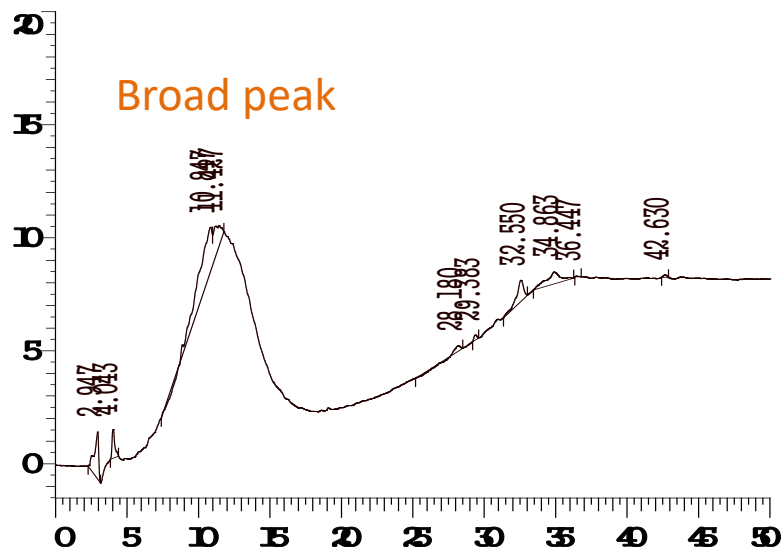
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# Analysis of the active fraction

## HPLC analysis



### HPLC condition

Column: InertSustain C18

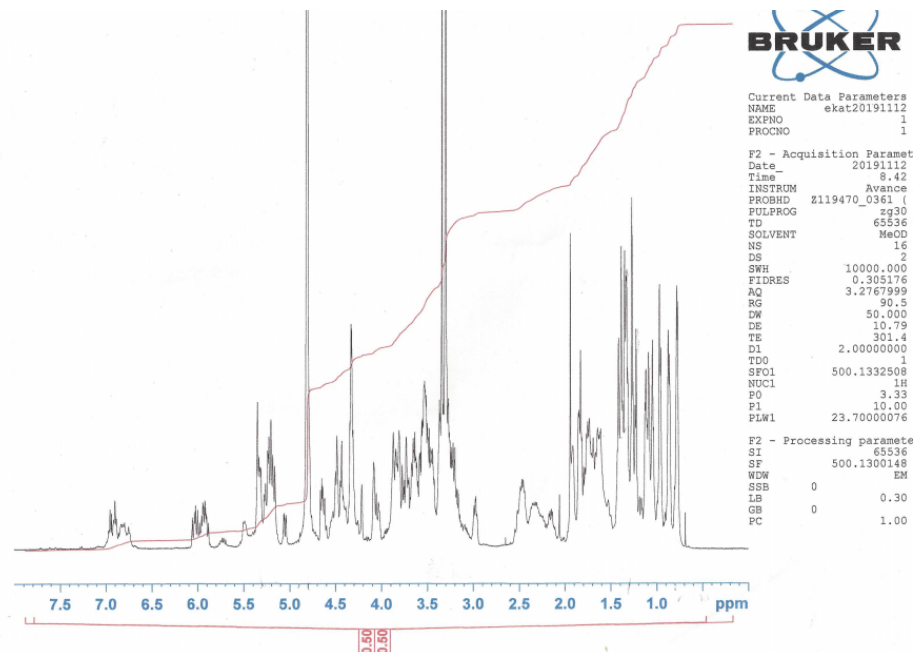
Eluent: 30%-85% aq. Acetonitrile+0.1% TFA(0-30min),

80% aq. Acetonitrile +0.1% TFA (30-50 min).

Flow: 1.0 mL/min.

UV: 254nm

## <sup>1</sup>H-NMR analysis



Saponins are suggested from the analysis

→ Structure analysis by decomposition



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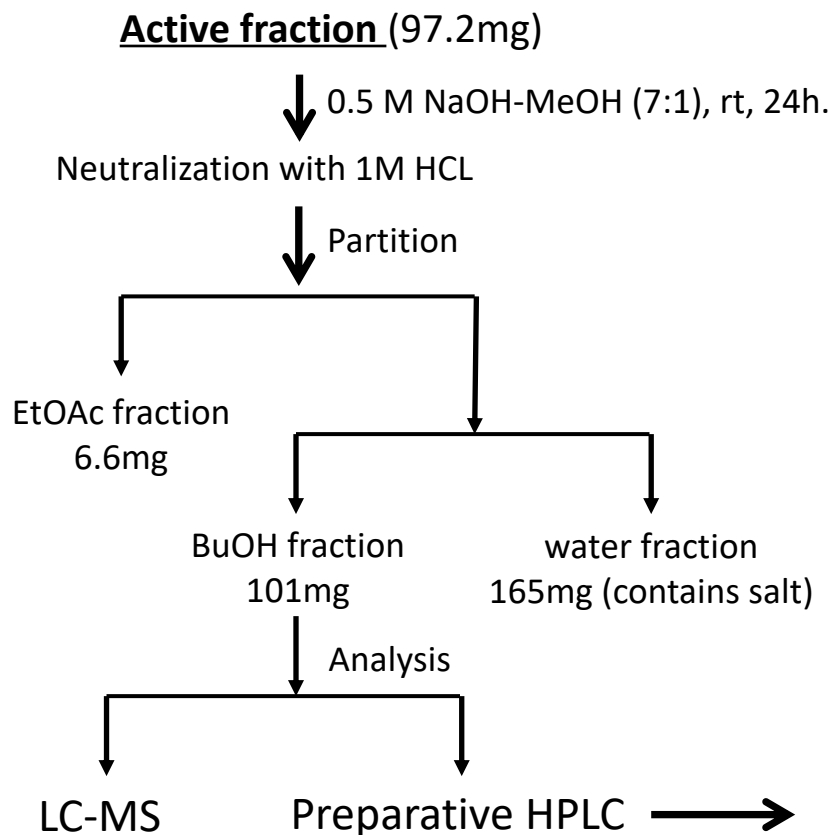
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# Structure analysis: decomposition by alkaline hydrolysis



## HPLC condition

Preparative HPLC

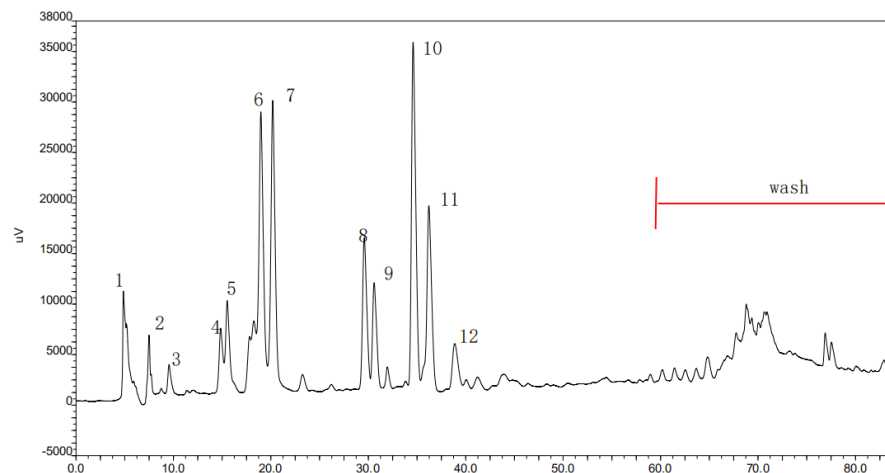
Column: InertSustain C18 (25 × 250 mm).

Eluent: 15%-35% aq. Acetonitrile+0.1% TFA(0-60min)

95% aq. Acetonitrile+0.1% TFA(60-90min)

Flow: 10.0 mL/min.

UV: 254nm



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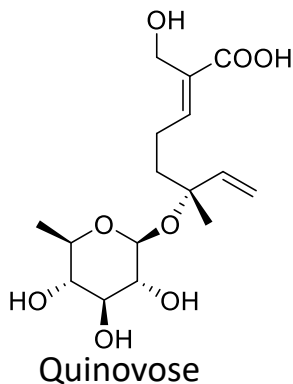
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# Structure analysis: decomposition by alkaline hydrolysis

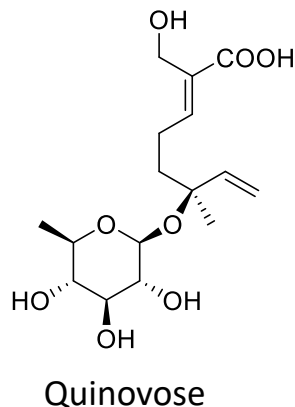
NMR analysis of compounds from butanol fraction of alkaline hydrolysis products

## Monoterpene Connected with sugar

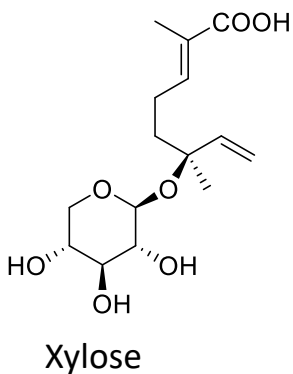
Peak 6 (*R*)



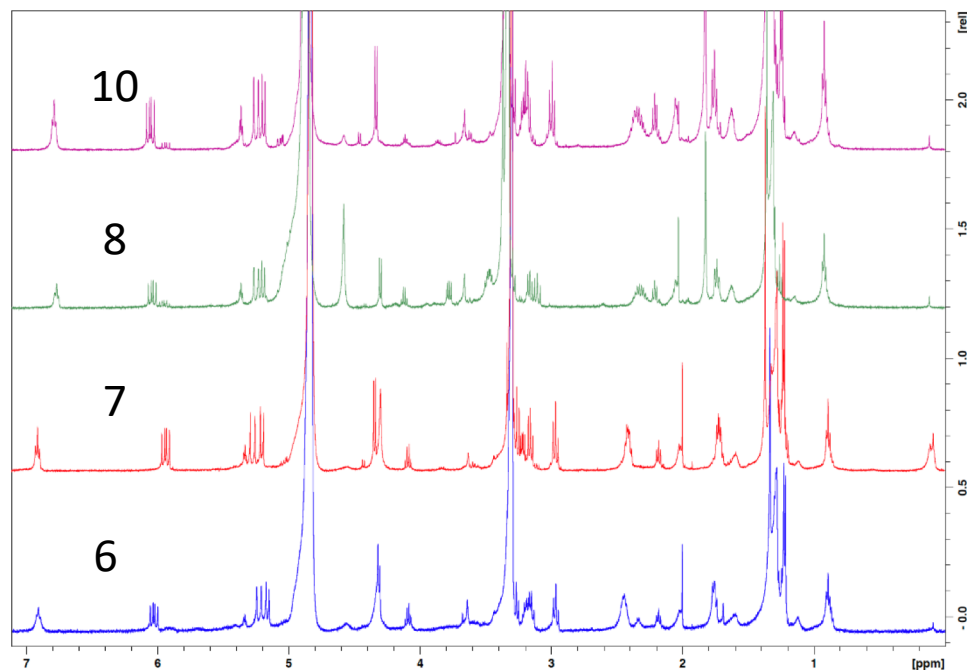
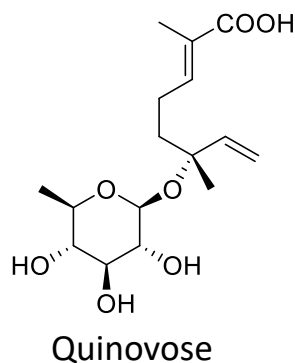
Peak 7 (*S*)



Peak 8 (*R*)



Peak 10 (*R*)



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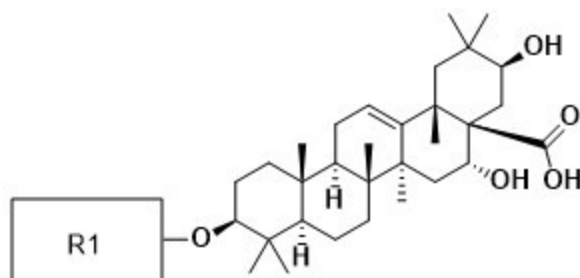
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# Structure analysis: decomposition by alkaline hydrolysis

LC-MS analysis of butanol fraction of alkaline hydrolysis products



13 prosapogenols → 3-O-glycosides of acacic acid or acacic acid lactone

R1	Acacic acid	Acacic acid lactone
Glu-Ara/Xylose	✓	✓
Glu-Rha	✓	✓
Ara-Rha	✓	✓
Glu-Glu-Glu	✓	-
Glu-Glu-Ara/xylose	✓	✓
Glu-Glu-Rha	✓	✓
Glu-Ara-Rha	✓	✓



# Structure analysis: decomposition by methanolysis

Active fraction (94.2mg)

↓ 1.25M methanolic HCL, 90°C, 1.5 hours

↓ Extraction by EtOAc

↓ PTLC separation

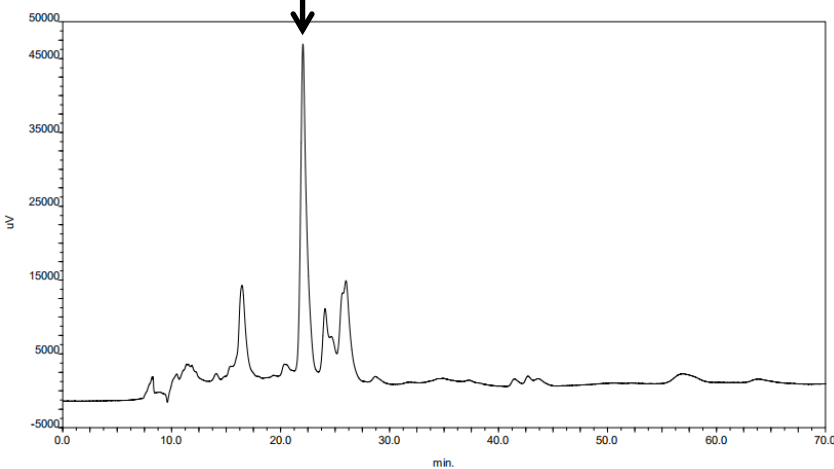
Reverse phase TLC  
separation

purified by HPLC

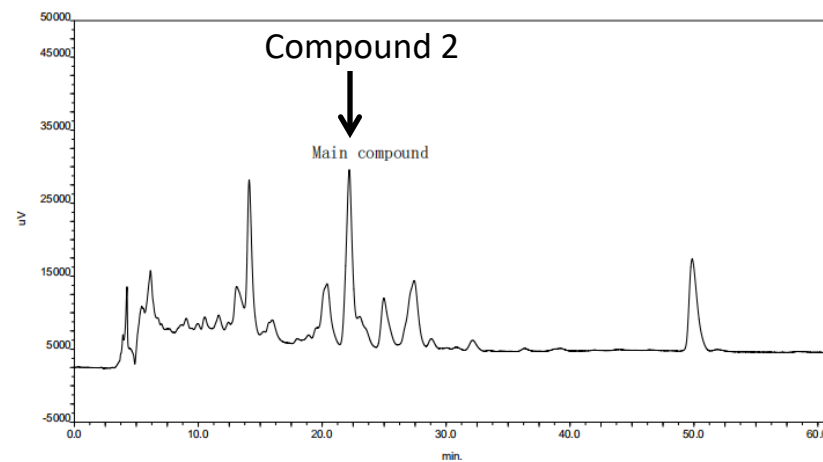
Compound 1

Compound 2

Compound 1



Compound 2



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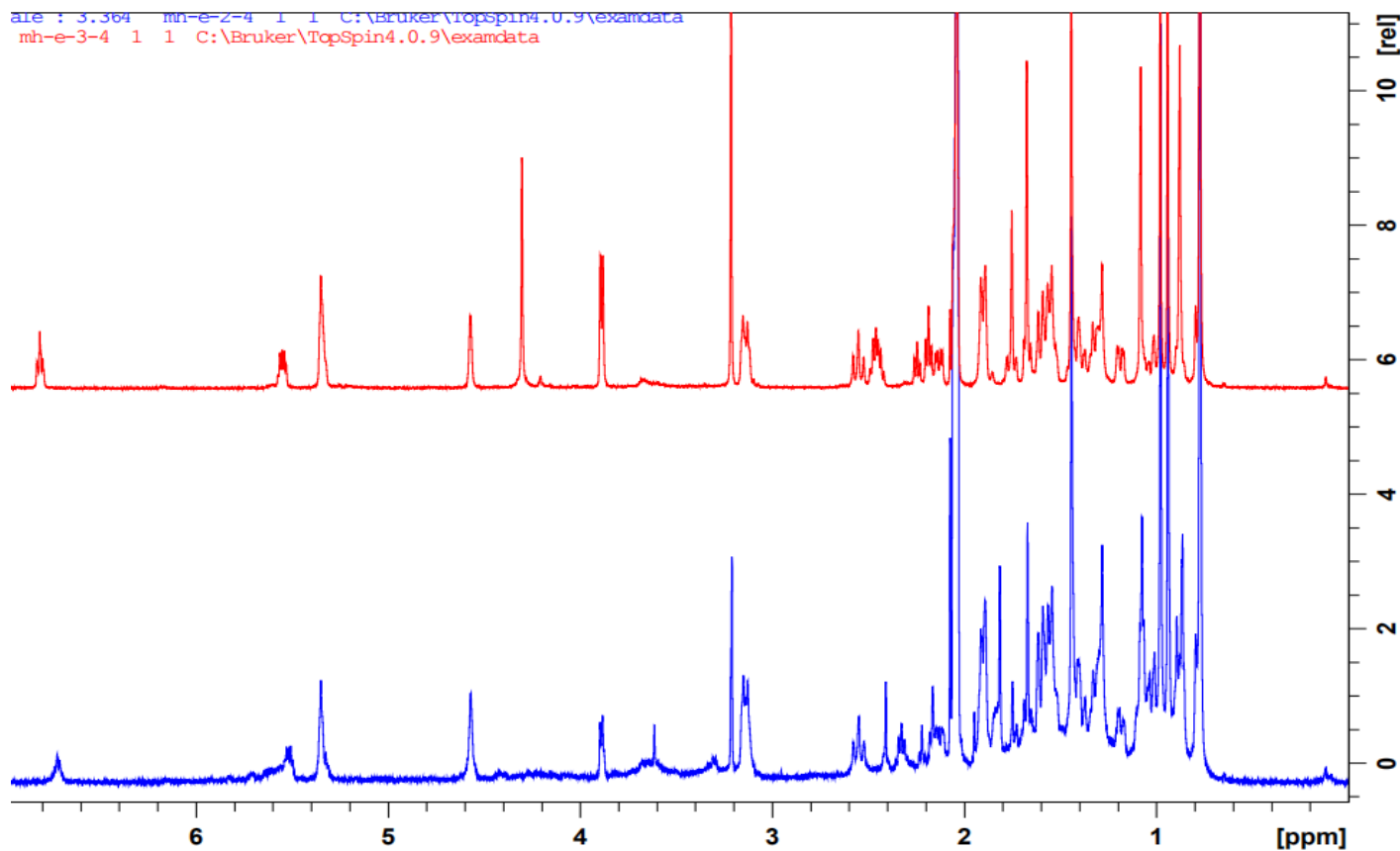
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# Structure analysis of compound 1,2: NMR analysis

Compound 1



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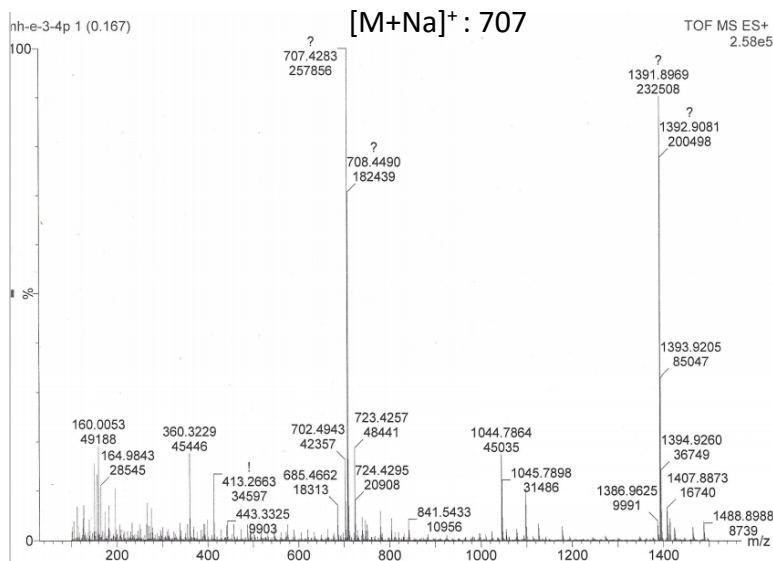
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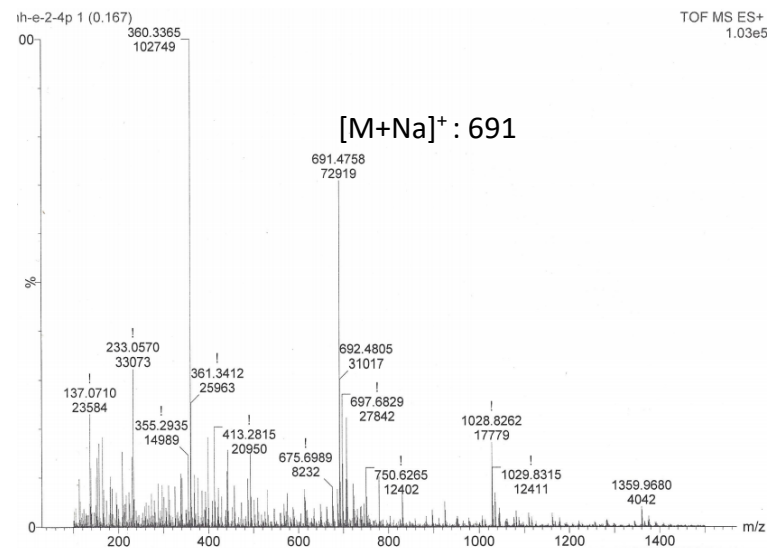
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# Structure analysis of compound 1,2: MS analysis

## Compound 1

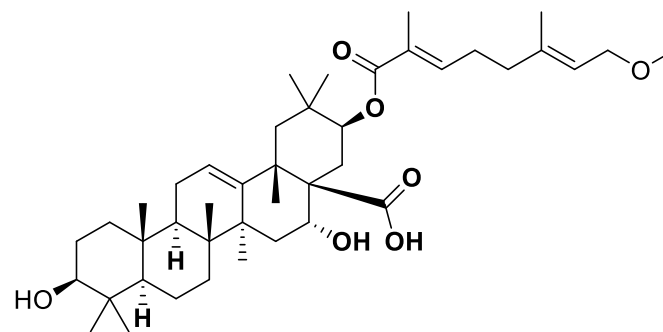
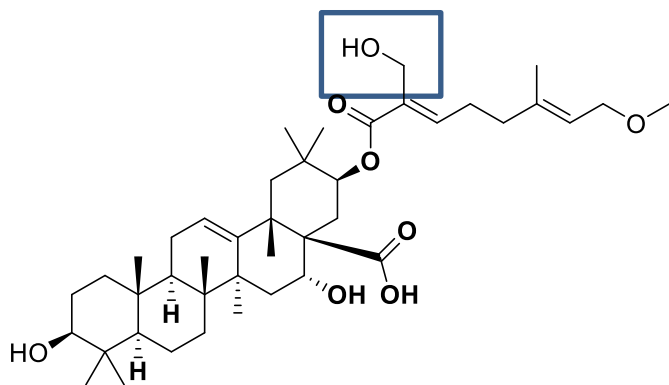


## Compound 2



HR-ESI-MS (positive): 684.4609 [M+H]<sup>+</sup>, C<sub>41</sub>H<sub>64</sub>O<sub>6</sub> calc. 668.4601

HR-ESI-MS (positive): 668.4665 [M+H]<sup>+</sup>, C<sub>41</sub>H<sub>64</sub>O<sub>7</sub> calc. 668.4652



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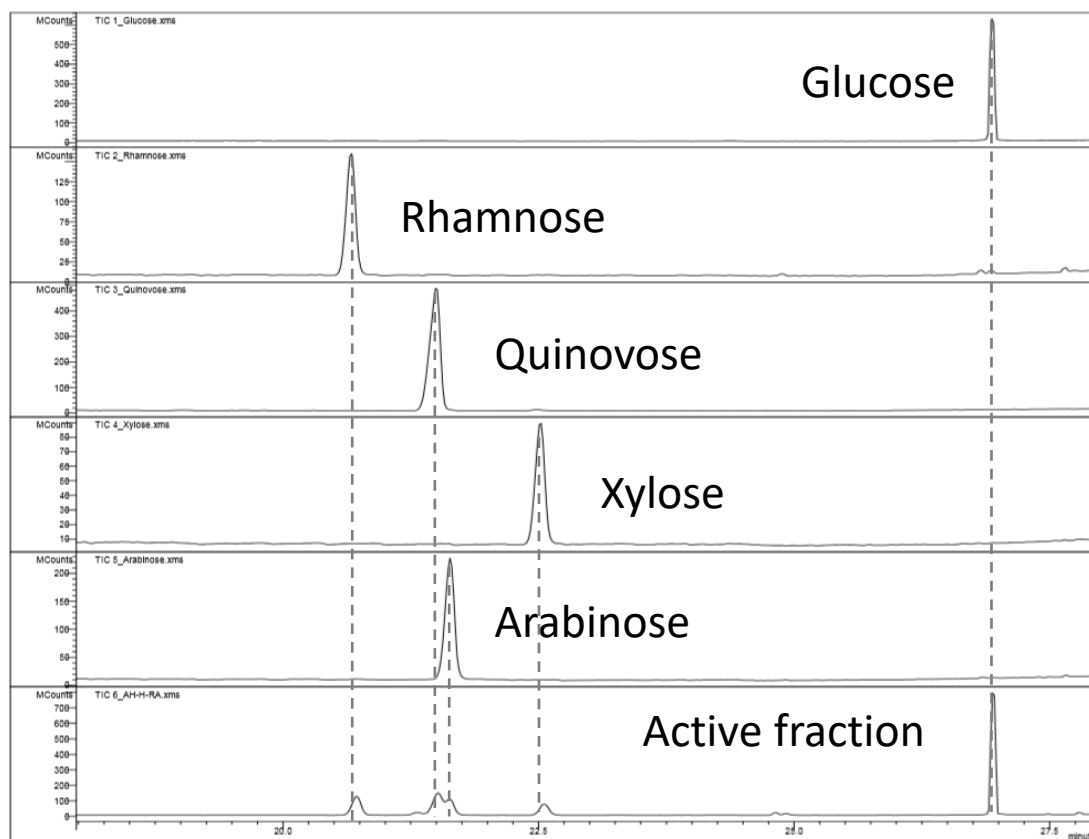
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# Structure analysis: sugar determination by GC-MS

**Pre-treatment**— Acidic Hydrolysis, Reduction and acetylation

- Hydrolysis: 1M HCl, 100°C, 6 hrs with reflux equipment
- Reduction: 10mg sample was reduced with 0.25M aq.NaBH<sub>4</sub> overnight
- Acetylation: React with 0.5 ml pyridine and 0.25 ml acetic anhydride with reflux equipment overnight, monitoring by TLC



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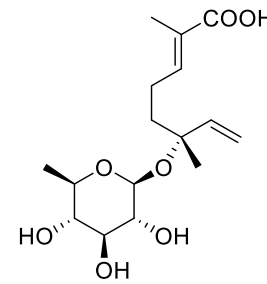
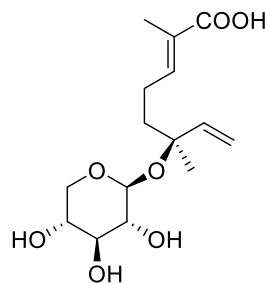
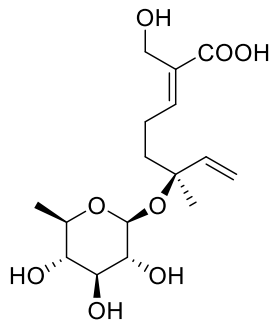
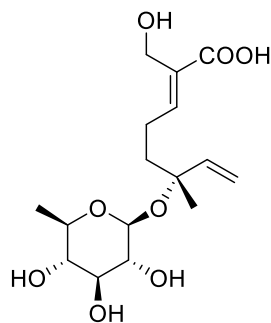


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# Short conclusion: structure analysis

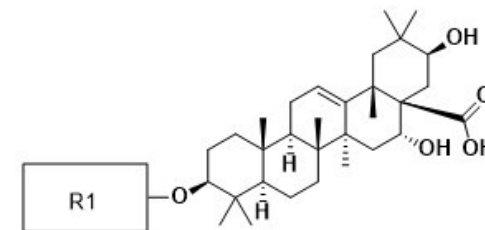
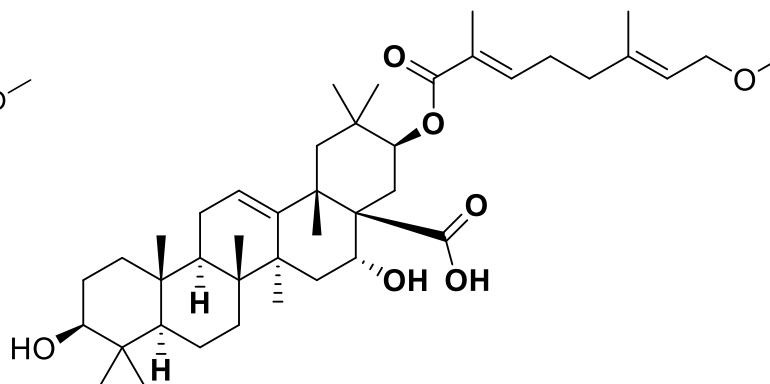
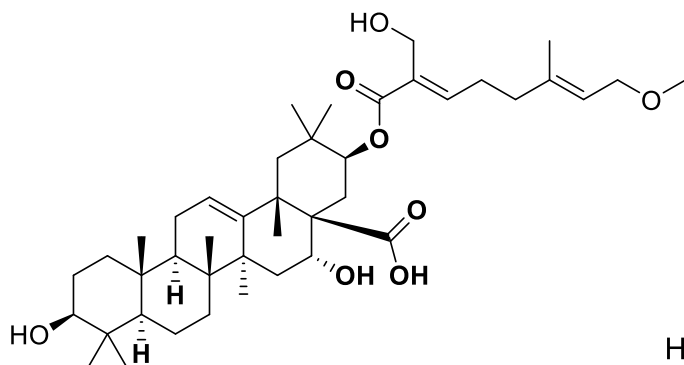
## 1. Alkaline hydrolysis

Monoterpenes connected with sugar (xylose and quinovose)



## 2. Methanolysis

triterpene connected with monoterpenes



## 3. Sugar components

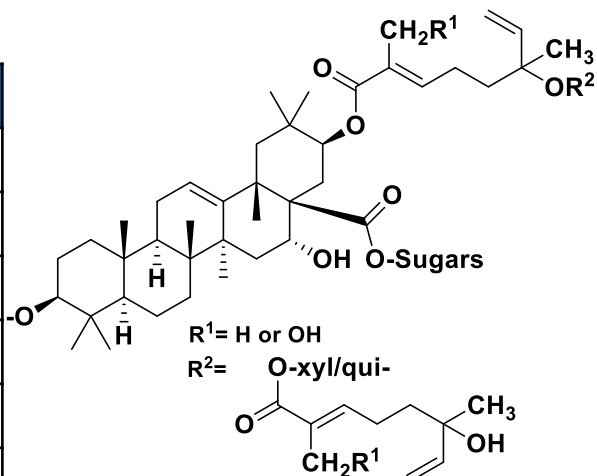
R1: glucose, rhamnose, quinovose, xylose, arabinose



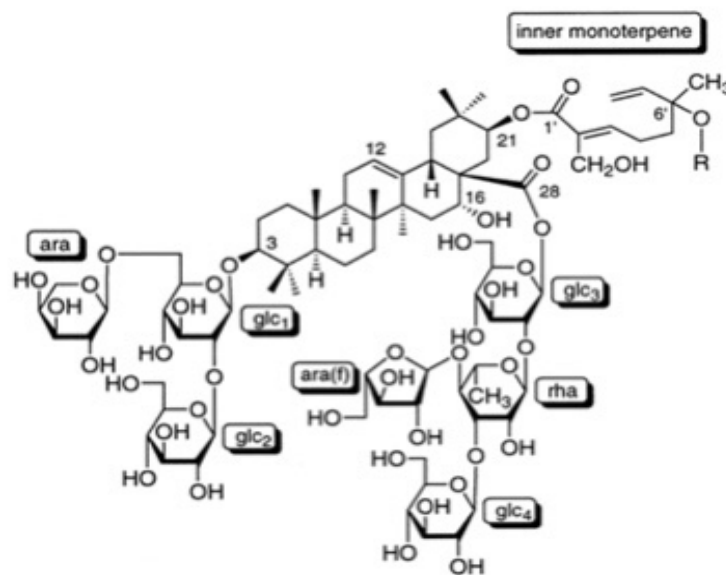
# Short conclusion: structure analysis

## Predicted structure of the active fraction

Sugars
Glu-Ara/Xylose
Glu-Rha
Ara-Rha
Glu-Glu-Glu
Glu-Glu-Ara/xylose
Glu-Glu-Rha
Glu-Ara-Rha



## Reported saponin from *A. concinna*



Tezuka *et al.* (2000). *Journal of Natural Products*, 63, 1658-1664.

## Evaluation of anti-obesogenic activity

- Pancreatic lipase inhibition
- Lipolysis enhancement



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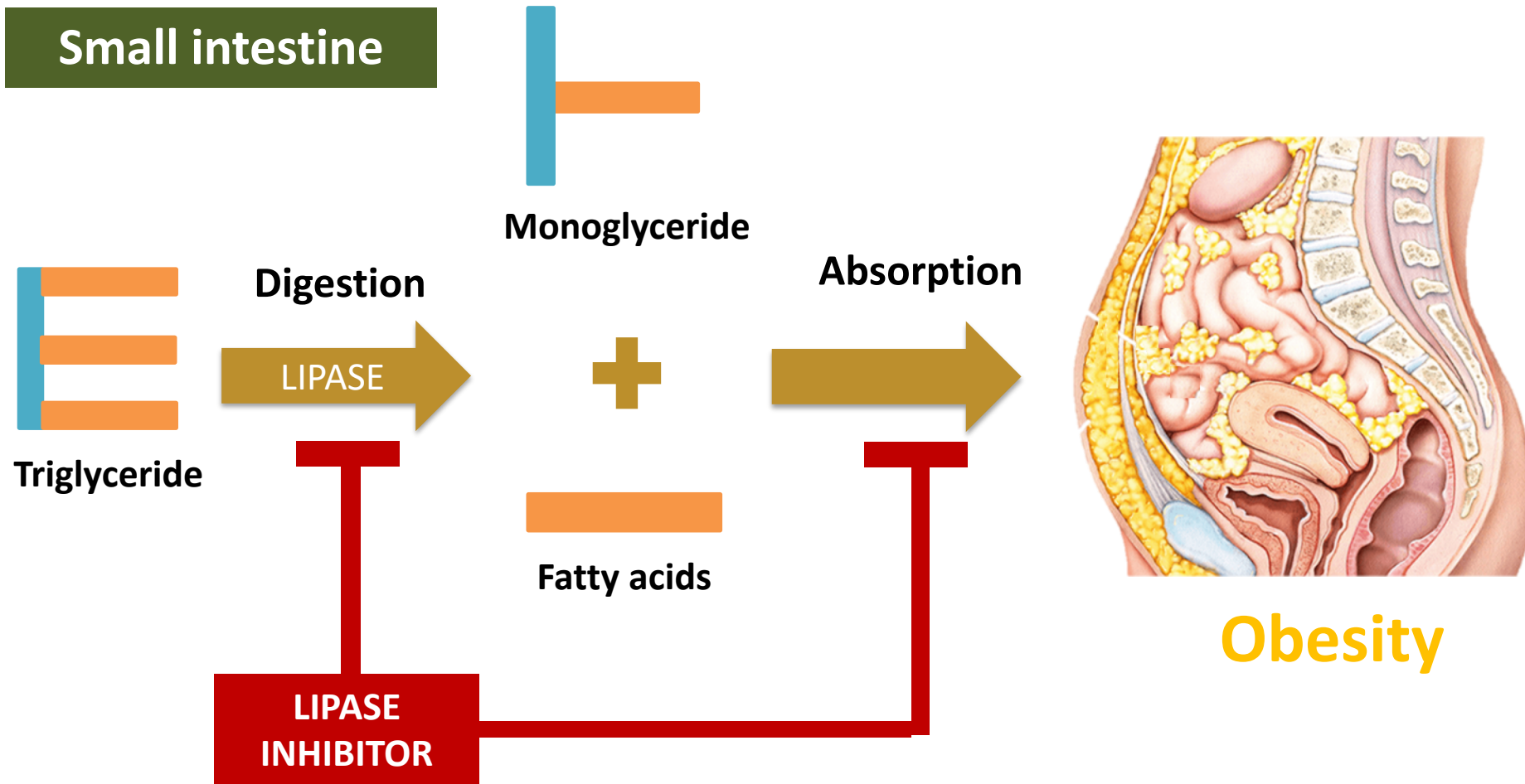
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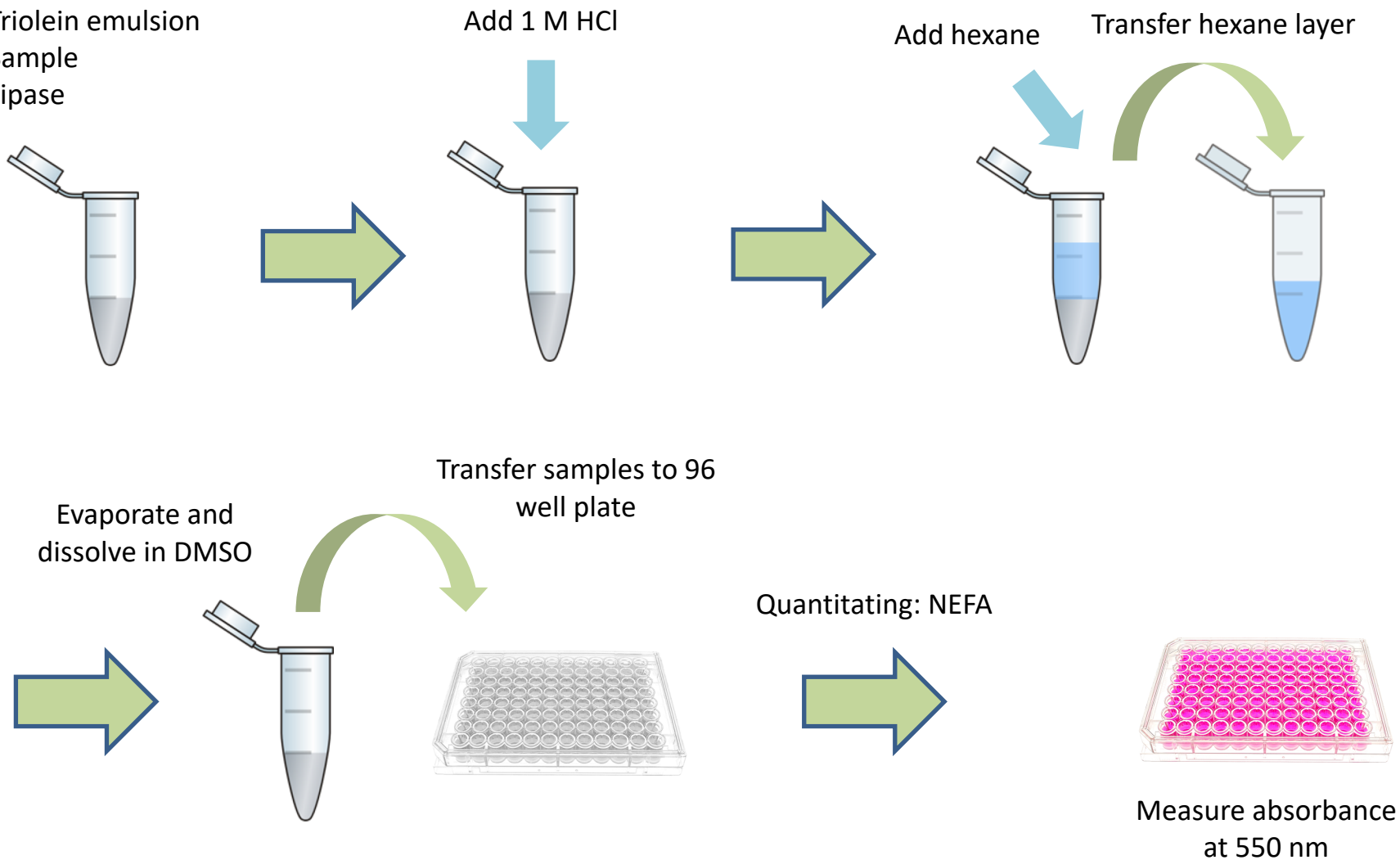
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# Pancreatic Lipase inhibition

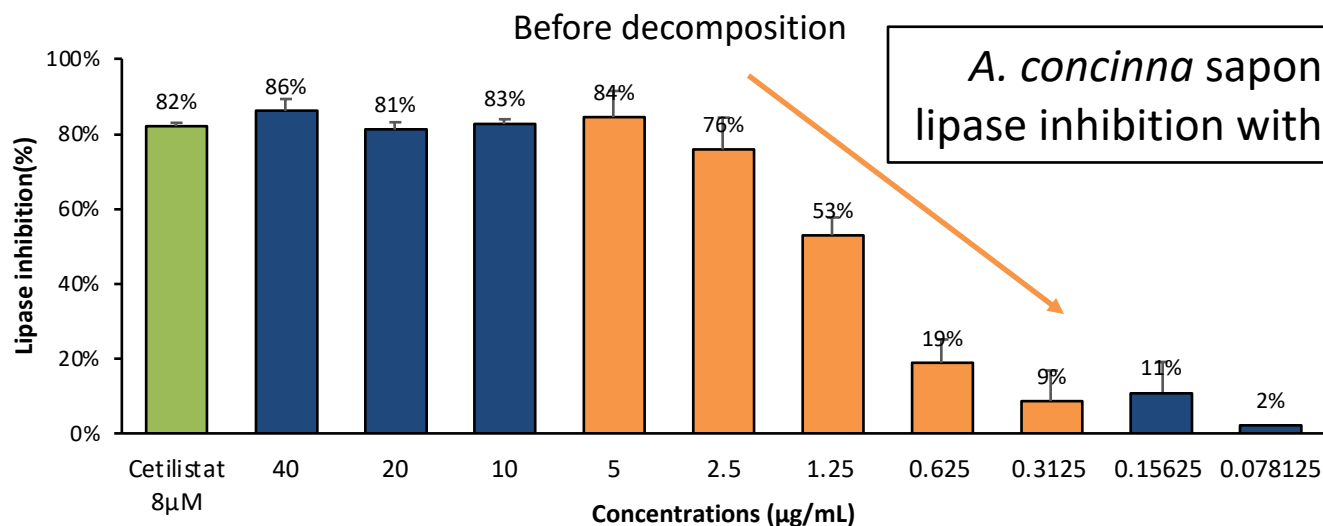


# Lipase inhibition: Method

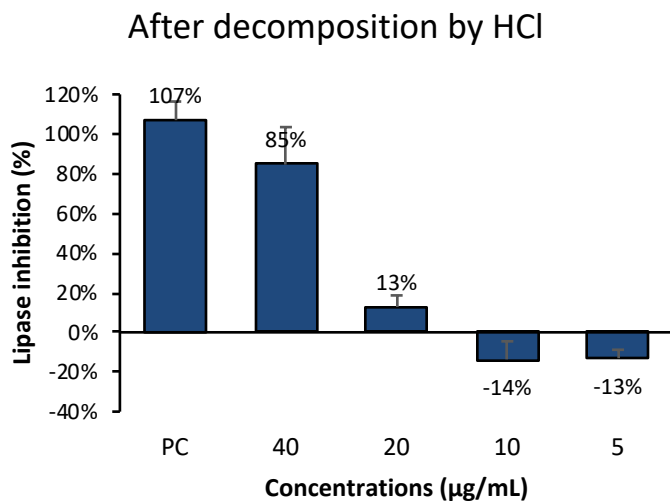
- Triolein emulsion
- Sample
- Lipase



# Lipase inhibition assay result



*A. concinna* saponin showed strong lipase inhibition with  $\text{IC}_{50} = 7.93 \mu\text{g} / \text{ml}$

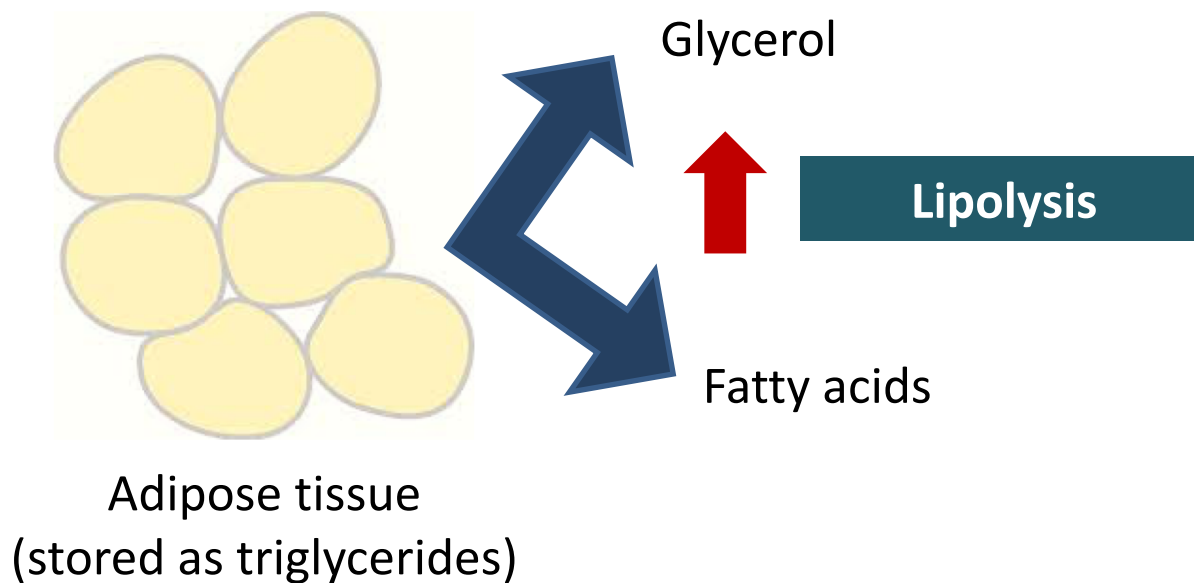


whole structure is important for the lipase inhibition

PC = active fraction before decomposition ( $30 \mu\text{g/mL}$ )



## 3T3-L1 adipocyte: Lipolysis

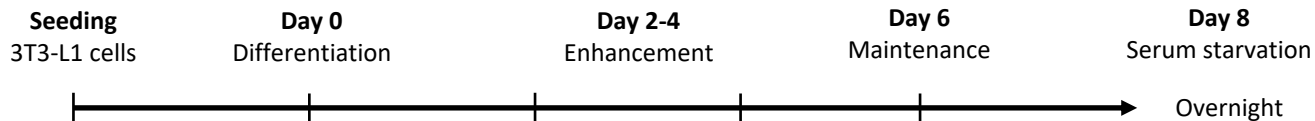


**Effective to treat obesity**



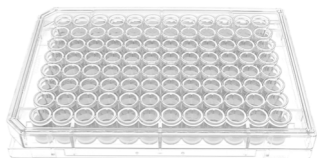
# 3T3-L1 adipocyte: Assay method

Cell culture



Glycerol release assay

Add: crude sample



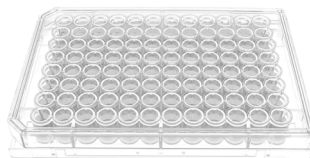
Incubate  
24 hrs



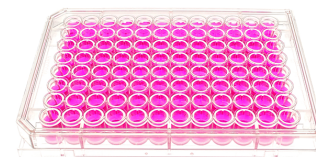
Supernatant  
transfer



Add glycerol reagent



Quantify:  
released glycerol



Measure absorbance  
at 540 nm



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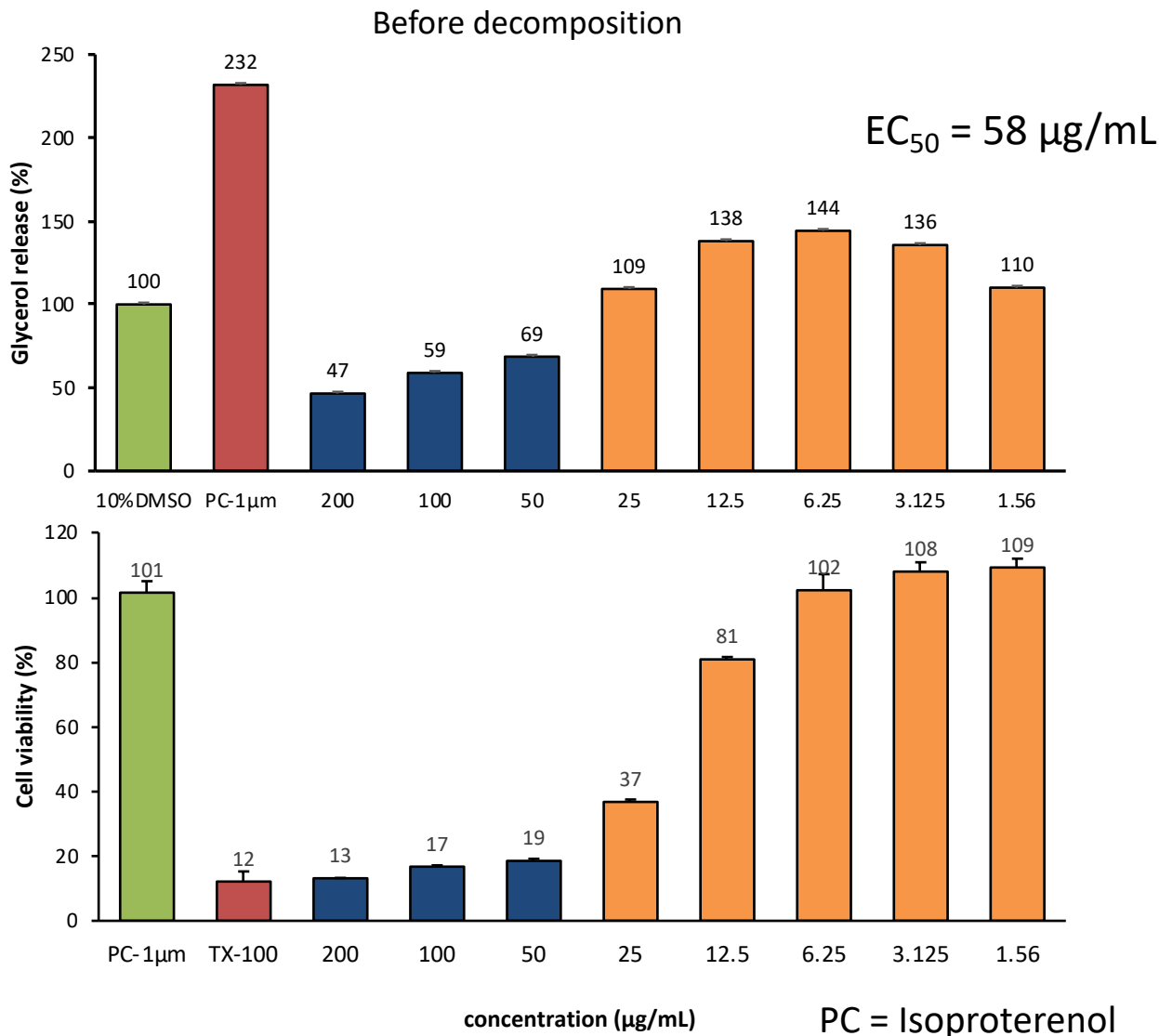
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# 3T3-L1 adipocyte: Enhancement of Lipolysis



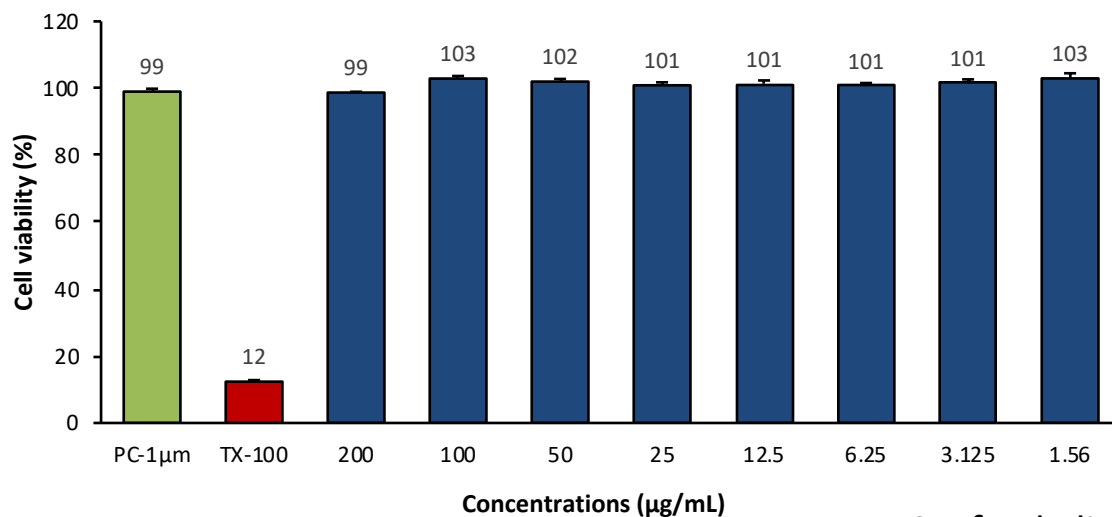
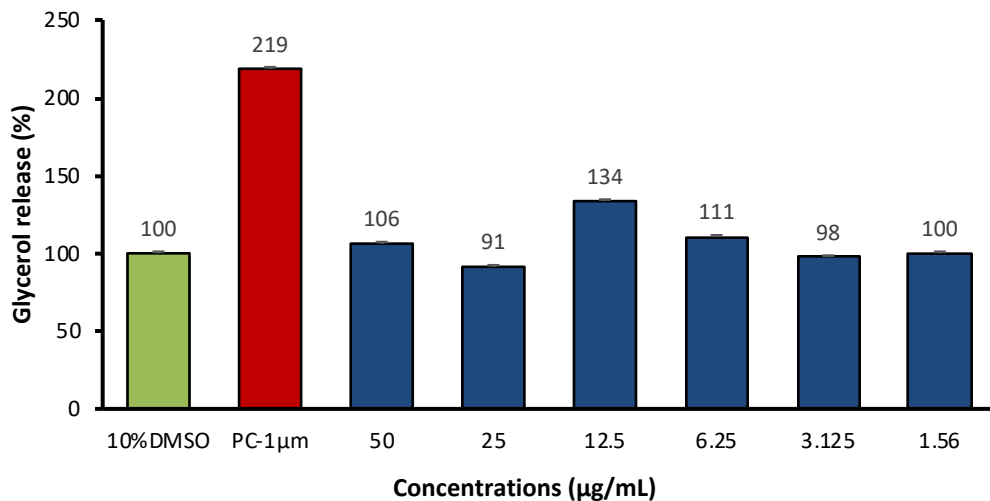
In the low concentration

- Enhanced lipolysis
- no cytotoxicity



# 3T3-L1 adipocyte: Enhancement of Lipolysis

After decomposition



PC = forskolin

Decomposition of saponin diminishes the lipolysis activity



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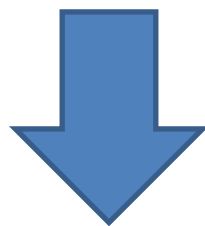
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## Conclusions

- *A. concinna* saponin was isolated as the major bioactive compound.
- *A. concinna* saponin is an efficient lipase inhibitor.
- The saponin also is a lipolysis enhancer in 3T3-Li adipocytes.



***A. concinna* saponin might be a good source for the treatment of obesity.**

