

## Supplementary materials

# Hypolipidemic activity of low-cholesterol ovum oil of *Rana chensinensis* and phytosterol (stigmasterol) in rats

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## Data S1 Materials and methods

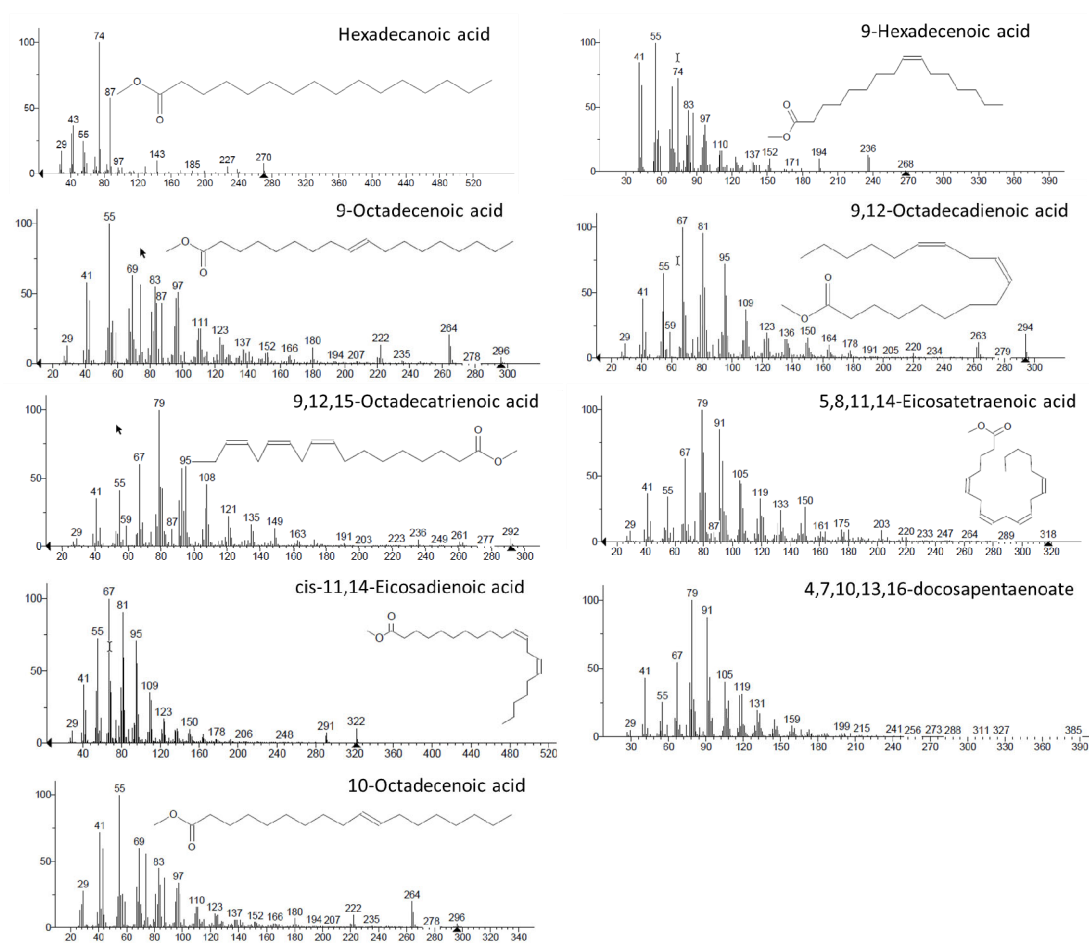
### Materials and chemicals

The OORC was extracted from the frog ovum powder by a series of processing. Briefly, the powder was filtered using 40 mesh, ethanol (app. 1:10 w/w) soaked for 3 h followed by microwave treatment (600W) for 30 min and then concentrated using a rotary evaporator. The yield was approximately 20%. Preparation and the oil composition of low-cholesterol OORC prepared using co-precipitation with beta-cyclodextrin ( $\beta$ -CD) was reported previously (Zhang et al., 2019). Briefly, the  $\beta$ -CD was dissolved in at 25 °C. The 10 ML 70% ethanol solution containing  $\beta$ -CD was added with 5 g of OORC and stirred for 3 h at 40 °C. Next, the solution was centrifuged at 3200 r/min for 15 min. The supernatant was collected for later use. The cholesterol content of OORC was approximately 3%, which was 60% less than its original content. The main fatty acids included hexadecanoic acid (17%), 9-Octadecenoic acid (23%), 10-Octadecenoic acid (2%), 9,12-Octadecadienoic acid (22%), 9,12,15-Octadecatrienoic acid (8%), 5,8,11,14-Eicosatetraenoic acid (8%), 9-Hexadecenoic acid(Z) (3%), cis-11,14-Eicosadienoic acid (1%), and 4,7,10,13,16-docosapentaenoate (4%), the structures were shown in Fig. 1. Simvastatin was provided from Yongjiaxian Sanjing Pharm group, Mingshui Pharmaceutical Co., LTD. The phytosterol (stigmasterol) was from Cofco Day Biological Engineering Co., LTD.

### Animal experiment

Sprague- Dawley rats (SPF  $N=64$ ; male, 190–200g in body weight) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. The rats were randomly divided into eight groups, each containing eight rats. The normal control group of rats were fed with the standard diet, while the

high fat diet induced hyperlipidemic group were fed with high fat diet containing 60% of calories from fat. Rats with various interventions were gavaged with different agents as shown in Table 1. The amount of phytosterol (stigmasterol) used in the current study was modified from the human report, which was based on 1.2 g/1000 kcal diet (Jenkins et al., 2003). The rats were acclimated to the new environment for one week before the experiment. Then the rats were fed for 5 weeks and then killed by decapitation under euthanasia of aether. The liver and spleen were taken out and weighted to calculate the organ indices as organ weight/body weight.



**Fig. 1 Structures of the main fatty acids in OORC**

**Table 1 Effect of low-cholesterol OORC and phytosterol on body weight of rats**

Group and treatments		Mean body mass (g)		
		0 weeks	2 weeks	5 weeks
Normal group	Physiological saline 4 mL/(kg·d)	263.55±2.85	358.25±8.47	409.19±8.97
Hyperlipidemic group	Physiological saline 4 mL/(kg·d)	262.74±3.61	373.97±10.59	451.13±23.68*
Positive control group	Simvastatin 3.33 mg/(kg·d)	264.13±1.08	379.47±4.56	476.01±18.55*
Phytosterol group	stigmasterol 0.48 g/(kg·d)	234.27±3.83	345.10±17.48	410.04±25.18 <sup>#</sup>
OORC group	OORC 2 g/(kg·d)	245.30±1.77	353.78±6.03	413.61±30.48 <sup>#</sup>
Low dose of OORC phytosterol group	OORC+stigmasterol 1+0.24 g/(kg·d)	240.85±2.58	345.95±12.75	409.95±9.91 <sup>#</sup>
Middle dose of OORC phytosterol group	OORC+stigmasterol 2+0.48 g/(kg·d)	244.15±7.49	338.04±31.89	404.35±21.75 <sup>#</sup>
High dose of OORC phytosterol group	OORC+ stigmasterol 4+0.96 g/(kg·d)	237.65±2.58	343.34±9.53	402.53±31.40 <sup>#</sup>

Note: \* Compared with the normal control group,  $P<0$ ; <sup>#</sup> Compared with the hyperlipidemic model group,  $P<0.05$ . Values are means±SD ( $n=8$ )

#### Statistical analysis

The figures were prepared using software Origin version 9.1. One-way ANOVA was performed with the data using SPSS, version 19.0. Multiple comparison was carried out using the least significant difference (LSD) test. The difference of values between different groups was considered to be significant when  $P<0.05$ .

#### References

- Jenkins, D.J.A., Kendall, C.W.C., Marchie, A., Faulkner, D., Vidgen, E., Lapsley, K.G., *et al.*, 2003. *The effect of combining plant sterols, soy protein, viscous fibers, and almonds in treating hypercholesterolemia*, **52**(11):1478-1483. [doi: [https://doi.org/10.1016/S0026-0495\(03\)00260-9](https://doi.org/10.1016/S0026-0495(03)00260-9)]
- Zhang, T., Zhao, C., Zhao, C., Zhou, X., Liang, Y. and Ye, H., 2019. *Optimization of conditions for cholesterol removal from Rana chensinensis ovum oil*, **17**(2):135-139.