*Journal of Food and Nutrition Research, 2014, Vol. 2, No. 10, 664-670*
Available online at http://pubs.sciepub.com/jfnr/2/10/2

DOI:10.12691/jfnr-2-10-2

# The Inhibitory Effect of Different Solvents Extracts from Walnut Shell (*Juglans regia L.*) on Pancreatic Lipase and Adipogenesis of 3T3-L1 Preadipocytes

**Jiong Yang[1], Chaoyin Chen[1,*], Shenglan Zhao[2,*], Feng Ge[1], Diqiu Liu[1]**

[1]Faculty of life science, Kunming University of Science and Technology, Kunming, People's Republic of China
[2]Yunnan University of Traditional Chinese Medicine, Kunming, People's Republic of China
*Corresponding author: chaoyinchen@163.com; zhaoshenglan@163.com

*Received September 02, 2014; Revised September 16, 2014; Accepted September 19, 2014*

**Abstract** In this article, the effect of the solvents (water, methanol, ethanol, chloroform, N-butanol and ethyl acetate) on the extraction yields (EY), total flavonoids content (TFC), total phenols content (TPC) form walnut shell and the inhibitory effect of extracts on pancreatic lipase were analysed. The kinetics of enzyme inhibition and was investigated. The inhibitory effect of extracts on adipogenesis of 3T3-L1 preadipocytes was also investigated. The highest EY, TFC and TPC were achieved with N-butanol (4.54%), Ethyl acetate (80.40 mg QEs/g extract) and ethyl acetate (200.40 mg GAE/g extract). The order of inhibitory activity was methanol extract (ME) > ethyl acetate extract (EAE) > ethanol extract (EE) > water extract (WE) > N-butanol extract (NBE) > chloroform extract (CE), the inhibition ratios were 82.51%, 73.13%, 70.49%, 69.42%, 65.57% and 60.66%, respectively under the concentration of 500 μg/mL. The $IC_{50}$ values of WE, ME, EE, CE, NBE and EAE were 371.35, 308.91, 407.57, 196.50, 247.79 and 254.27 μg/mL respectively. The inhibitory types of walnut shell extracts were noncompetitive. All the extracts were non-toxic to 3T3-L1 preadipocytes. The ME, EE, CE, NBE and EAE have inhibitory effect on triglyceride accumulation of 3T3-L1 cells.

***Keywords:*** *walnut shell, solvents extraction, pancreatic lipase, obesity*

**Cite This Article:** Jiong Yang, Chaoyin Chen, Shenglan Zhao, Feng Ge, and Diqiu Liu, "The Inhibitory Effect of Different Solvents Extracts from Walnut Shell (*Juglans regia L.*) on Pancreatic Lipase and Adipogenesis of 3T3-L1 Preadipocytes." *Journal of Food and Nutrition Research*, vol. 2, no. 10 (2014): 664-670. doi: 10.12691/jfnr-2-10-2.

## 1. Introduction

Prevalence of obesity is becoming one of the greatest threats to global health owing to the people's lifestyle of decreased physical activity and intake of high energy food. Obesity not only affects human appearance, but also causes many diseases, such as hepatic adipose infiltration [1], arteriosclerosis [2], hypertension, coronary heart disease, type II diabetes and other chronic diseases [3]. Due to the increasing obesity rates, the World Health Organization has prompted to promote strategies to prevent and control its progress [4]. Moderate weight loss by lifestyle changes improves obesity which is the main cornerstone of the therapy for obesity. Unfortunately, this clinical approach is not-long term lasting and weight regain is often seen, so the drugs are necessary to prevent weight regain [5].

Dietary triglycerides are hydrolyzed by lipase to monoglycerides, fatty acids and other small molecules, which are absorbed and resynthesized triglycerides leading to obesity ultimately [3]. The inhibition of fat digestion is an approach for reducing lipid absorption which can reduce the chance of obesity. Pancreatic lipase (PL) which can catalyze the hydrolysis of dietary triglycerides is the key enzyme for lipid absorption. So it is an effective way to prevent obesity by inhibition the activity of lipase in the digestive organs. Orlistat is the hydrogenated derivative of lipstatin, an inhibitor of PL isolated from the *Streptomyces toxytricini* and the only authorized anti-obesity drug in Europe. The drug has effective for the treatment of obesity, but side effects, such as fecal incontinence, have also arisen [4]. Therefore, researches need to find natural anti-obesity compound to avoid the side effects. Now, a lot of extracts from hundreds of species of plants [6] and marine algae [7] as well as the metabolites from microorganisms [8] are being screened for their lipase inhibitory activity. In the research of effective antiobesity agents, several categories of natural compounds such as polyphenols, saponins, triterpenes, flavonoids, some certain proteins and basic polysaccharides show potent inhibitory activity against PL [4].

3T3-L1 mouse preadipocytes are known to differentiate into mature adipocyte cells in vitro and intracellular accumulation of lipid droplets has been observed in cell differentiation. Some natural products and plant extracts were found to inhibit the adipogenesis of 3T3-L1 cells [9,10].

Walnut shells are an abundant agricultural residue generated in the walnut harvest which can be valued as a source of natural compounds. They are good media to separate crude oil from water [11]. The shell also can be used as a carbonaceous sorbent to control metal from aqueous solution [12]. Walnut shell has many natural compounds such as phenols and flavonoids [13] which have potential inhibitory effect on pancreatic lipase.

The aim of this work was to analyse the effect of the solvent on the inhibitory effect on PL of walnut shell extracts. Solvents (water, methanol, ethanol, chloroform, N-butanol and ethyl acetate) with different polarity were used. The extracts were studied, regarding their extraction yield, total flavonoids content, phenols content and PL inhibitory activity. The influence of pH and reaction time on inhibitory effect and kinetics of inhibition against PL were investigated. The inhibitory effect of extracts on adipogenesis of 3T3-L1 preadipocytes was also investigated.

# 2. Materials and Methods

## 2.1. Chemicals and Reagents

Sodium hydroxide (NaOH), polyvinyl alcohol (PVA), aluminum nitrate [$Al(NO_3)_3$], sodium nitrite ($NaNO_2$), sodium carbonate ($Na_2CO_3$), sodium dihydrogen phosphate ($NaH_2PO_4$), disodium hydrogen phosphate ($Na_2HPO_4$), olive oil and Folin-Ciocalteau's phenol reagent were provided from Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd. (Tianjin China). Quercetin, orlistat, gallic acid (GAE), DMSO, insulin, 3-isobutylmethoxylxanthine (IBMX), dexamethasone and porcine pancreatic lipase (PL) were purchased from Sigma (China). Penicillin-streptomycin and DMEM were obtained from Gibco (China). Ethyl acetate, methanol, 95% ethanol, chloroform and N-butanol were obtained from Chongqing Chuandong Chemical group Co., Ltd. (Chongqing China).

## 2.2. Preparation of Raw Material

The sampling of raw material was following the method described by Fernández-Agulló [14] with some modifications. Walnut shells were gathered from Zhaotong city in Yunnan province of China. Prior to the use, the walnut shells were cleaned of dirt and impurities, and then ground into powders using a grinder. The powders were passed through a 60-mesh screen. To preserve chemical properties, the particles were stored in plastic bags at -70℃, and then freeze dried for further use.

## 2.3. Extracts Preparation

The extracts were following the method described by Fernández-Agulló [14] with some modification. For the water extractions, 50 g of powdered sample with a ratio 1:30 (w/v) were extracted with boiling water for 45 min and filtrated through Whatman no.4 paper. In the extractions with methanol, ethanol, chloroform, ethyl acetate and N-butanol, 50 g of simple were extracted with 1500 mL of tested solvent for 45 min at 40 ℃ and filtered through Whatman no.4 paper. The filtrates were evaporated under vacuum in a rotary evaporator then the concentrates were frozen, lyophilized and redissolved in water at concentrations of 1 mg/mL for further use. All the extractions were done in triplicate.

## 2.4. Total Flavonoids Content

The TFC in the obtained extracts was determined by colorimetric assay [15] with a little modification. Briefly, 1 mL sample ethanol solution was mixed with 0.3 mL $NaNO_2$ aqueous solution (5%, w/w). The mixture stood for 6 min, followed by the addition of 0.3 mL $Al(NO_3)_3$ aqueous solution (10%, w/w). After deposing for 6 min, 4 mL NaOH aqueous solution (4%, w/w) was added and the volume was adjusted to 10 mL with distilled water. After incubation for 30 min at room temperature, the absorbance was determined at 508 nm. The results were based on the calibration curve with quercetin. The quercetin was dissolved in ethanol solution, and the concentrations of quercetin for the standard curve were 0-500 μg/mL. The TFC was calculated as a quercetin equivalent and expressed as mg quercetin equivalents (QEs) mg/g of extract. The determinations were performed in triplicate.

## 2.5. Total Phenols Content

The TPC was determined using the Folin-Ciocalteau assay accordingly to Ghasemzadeh [16] with some modifications. Briefly, 1 mL of extract was mixed with 1 mL of Folin-Ciocalteau's reagent. After 5 min, 2 mL of $Na_2CO_3$ solution was added to the mixture and adjusted to 10 mL with distilled water. After being kept in the dark for 60 min, the absorbance at 750 nm was measured. The total phenols content was calculated using GAE calibration curve of GAE standard solutions (0-500μg/mL) and expressed as μg GAE equivalents (GAE) μg/g of extract. The determinations were performed in triplicate.

## 2.6. Determination of Pancreatic Lipase Activity

The PL activity was measured by the classical olive oil emulsion method [17] described by Zhang with some modification. The substrate (5 mL) and PBS (4mL, pH 7.5) was mixed and preheated in water bath at 37 ℃ for 10 min. Then 1 ml of enzyme solution dissolving by PBS was added to the mixture to catalyze the hydrolysis reaction for 15 min and the reaction was terminated by adding 15 mL 95% ethanol. Blank samples were prepared in parallel before the addition of the enzyme. The amount of fatty acid produced was quantified by the volume of the consumption of NaOH (0.1 mol/L). One lipase unit was defined as the amount of enzyme needed to release 1 μmol fatty acid per minute under the experimental conditions. PL activity was computed from the following equation:

$$\text{PL activity} = [1000 \times (A - B) \times M] / TW$$

where A is the volume of experimental group consumed NaOH (mL), B is the volume of blank group consumed NaOH (mL). M is the molar concentration of NaOH solution (mol/L), T is the reaction time (min) and W is the mass of PL (g).

## 2.7. The Influence of Extracts Concentration on Inhibitory Effect

1 mL of each extract (100-500 μg/mL) was added to the mixture to investigate the PL inhibition as the method to determination of pancreatic lipase activity. The inhibitory effect of extracts on PL was obtained from:

$$\text{Inhibition rate} = [(A_1 - A_2) / A_1] \times 100\%$$

where $A_1$ is the PL activity and $A_2$ is the PL activity after adding extracts.

The concentration which inhibits 50% enzymatic activity ($IC_{50}$) was concluded from the graph of inhibition rate against the samples concentration.

## 2.8. Kinetics of Enzyme Inhibition

In order to clarify the kinetics of inhibition against PL, a series of substrate concentrations (0.0067, 0.0133, 0.0200, 0.0266, 0.0333 mg/mL) were tested in the assay system and each analysis was performed with or without the extracts. The inhibitory types were determined by Lineweaver-Burk plots, the inhibition constant ($K_i$) was calculated from the following equation:

$$K_{m,app} = K_m \left[ (1 + M) / K_i \right]$$

where $K_{m,app}$ and $K_m$ are the $K_m$ with or without the extracts, the M represents the concentration of the extracts [18].

## 2.9. 3T3-L1 Cell Culture and Adipocytes Differentiation

The 3T3-L1 Cell culture and adipocytes differentiation was used the method described by Vaidya [19] with some modifications. 3T3-L1 mouse pre-adipocytes were cultured (37 ℃, 5% $CO_2$) in DMEM containing with 10% FBS until reaching confluence. Then cells were stimulated with differentiation medium supplemented with DMEM with FBS (10%), insulin (10 μg/mL), dexamethasone (0.25 mM), IBMX (0.5mM). After 48 h, the cells maintained in DMEM containing FBS (10%) and insulin (10 μg/mL) for another two days. Finally, the cells cultured with DMEM with 10% FBS for an additional four days. All media contained 100 μg/mL streptomycin and 100 IU/mL penicillin. The cultures were treated with extracts for general experiments throughout the culture period.

## 2.10. Cell Viability and Triglyceride Assays

3T3-L1 cells viability was determined by the MTT assay [20] with some modifications. Cells incubated in 96 well microtiter plates ($1 \times 10^4$ cells/well) for 24 h. Then cells stimulated with 300 μg/mL of each extract for 24 h and MTT solution (5 mg/mL) was added to well. After 2 h of incubation at 37 ℃, the MTT reagent was removed. The formazan product was dissolved by the addition of 200 μL of DMSO. The absorbance was detected at 595 nm by use of a microplate reader, and cell viability was expressed as a percentage over control. Triglycerides were quantified using the Infinity Triglyceride kit (Thermo Scientific) [21]. Triglyceride was expressed as relative contents using a percentage over control.

## 2.11. Statistical Analysis

All the experiments were carried out in triplicate. The results are expressed as mean values and standard error of the mean. The existence of significant differences among the results fore extraction yield, total flavonoids content total phenols content and PL inhibitory properties of the extracts was analyzed. The difference was considered significant at $P \leq 0.05$. The one-way analysis of variance (ANOVA) and Duncan's test were used. All statistical tests were used SPSS 19.0 software.

# 3. Results and Discussion

Extraction with solvents is frequently used for the isolation of bioactivity compounds. Organic solvents are commonly used for the extraction of polyphenols and flavonoids [22,23,24] from plant material. Both the extraction yield and biological activity of the extracts have a strong relationship with the solvent employed, mainly due to the different polarity of the compounds obtained [25]. For this reason, different solvents (water, methanol, ethanol, chloroform, N-butanol and ethyl acetate) were assayed for their extraction of walnut shell in this work. The extraction yield, total flavonoids content, total phenols content and PL inhibitory properties of the extracts obtained was compared.

## 3.1. The Extraction Yield, Total Flavonoids Content and Total Phenols Content

Table 1 shows the results obtained for the extraction yield, total flavonoids content and total phenols content. The EY, TFC and TPC of various solvents used for the extraction of walnut shell showed significantly different ($p<0.05$).

**Table 1. Extraction yield (in percentage) and total flavonoids content (TFC mg QEs/g extract) and total phenols content (TPC mg GAE/g extract) of walnut shell from Zhaotong city in Yunnan province of China**

| Solvent | EY (%) | TFC (mg QEs/g extract) | TPC (mg GAE/g extract) |
|---|---|---|---|
| Water | 2.63 ±0.08c | 4.73 ±0.15a | 26.36 ±0.55c |
| Methanol | 3.20 ±0.08d | 48.90 ±0.79e | 27.58 ±1.24c |
| Ethanol | 1.87 ±0.14b | 9.38 ±0.40b | 23.86 ±1.18b |
| Chloroform | 0.42 ±0.01a | 14.89 ±0.43c | 10.76 ±0.15a |
| N-butanol | 4.54 ±0.20e | 30.72 ±0.35d | 179.48 ±4.45d |
| Ethyl acetate | 1.85 ±0.06b | 80.40 ±0.55f | 200.40 ±0.70e |

Data are means of three replicates with standard errors. Values in the same column followed by a different letter are significantly different ($p<0.05$).

The EY depended on many factors such as temperature [26], solid-liquid ratio [27] and the polarity of the solvent. Normally, the high temperature and solid-liquid ratio got high extraction yield. So in this work, a higher temperature and solid-liquid ratio were adopted. The values of the EY varied from 0.42% for the Chloroform extract to 4.54% for the N-butanol extract. The EY decreasing in the following order: N-butanol > methanol >

water > ethanol > ethyl acetate > chloroform. The results were similar with Sahreen's, the ME had a higher extraction yield [28].

The $NaNO_2$-$Al(NO_3)_3$ method and Folin-Ciocalteau assay used for determination of the total flavonoids and phenols content has been employed for many years. The total flavonoids content and total phenols content in walnut shell different solvents extracts were significantly different ($p<0.05$). The EAE showed the highest amount of these compounds, with 80.40 mg REs/g extract and 200.40 mg GAE/g extract. The total flavonoids content was higher than those results obtained by Akbari [13] but lower than the results got by Li [15] and Chaleshtori [29]. The total phenols content was higher than the results obtain by Ghasemzadeh [16]. The WE and CE showed the lowest content of flavonoids (4.73 mg REs/g) and phenols (10.76 mg GAE/g extract). The difference among TFC and TPC was mainly due to the polarity of the solvent and the solubility of extract in solvent.

## 3.2. Pancreatic Lipase Inhibitory Activity

A lot of reports have been published about the inhibitory effect of extracts against the pancreatic lipase. Liu and his co-workers [30] reported that the $IC_{50}$ value of *Nelumbo nucifera* leaf flavonoids on PL was 380 μg/mL. Gondoin [31] proved that white and green tea polyphenols had strong inhibitory effect on PL. Moreover very little research has been reported about the PL inhibition activity of walnut shell extracts. Walnut shells are waste generated in walnut (*Juglans regia* L.) harvest which contains natural compounds (flavonoids, phenols) with inhibitory effect on pancreatic lipase. So the inhibition activity of walnut shell on pancreatic lipase was investigated.

The inhibitory effect of the extracts against pancreatic lipase was determined using different concentrations (100-500 μg/mL) of extracts. As shown in Figure 1, the extracts had moderate inhibition of PL in a dose-dependent manner. The order of inhibitory sequences was ME > EAE > EE > WE > NBE > CE, the inhibition ratios were 82.51%, 73.13%, 70.49%, 69.42%, 65.57% and 60.66%, respectively under the concentration of 500 μg/mL. When the concentration of orlistat > 400 μg/mL, the inhibition rate reaches 100%. Nevertheless, the extracts still showed a strong inhibitory activity of pancreatic lipase compared with quercetin, isoquercitrin and rutin [3], especially ME. To some extent, The $IC_{50}$ values showed the inhibitory effect of inhibitors on enzyme active under a particular substrate concentration. The smaller of $IC_{50}$ value indicated the stronger inhibition. The $IC_{50}$ values of WE, ME, EE, CE, NBE and EAE were 371.35, 308.91, 407.57, 196.50, 247.79 and 254.27 μg/mL respectively. The $IC_{50}$ values were smaller than the results obtained by Marrelli [32] and You [33]. The walnut shell extracts can be as a potential medicine to treatment of obesity.

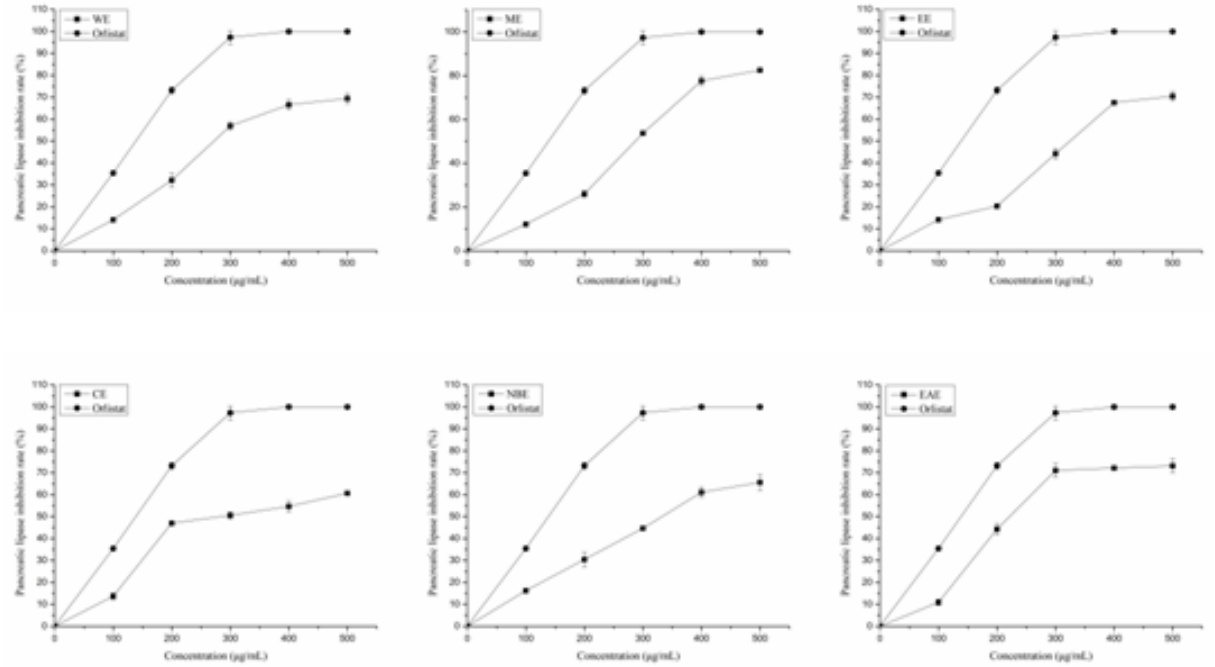


**Figure 1.** Effect of orlistat, water extract (WE), methanol extract (ME), ethanol extract (EE), chloroform extract (CE), N-butanol extract (NBE) and ethyl acetate extract (EAE) on pancreatic lipase activity

## 3.3. Kinetics of Enzyme Inhibition

Many reports have been published about the inhibitory activity of plant material against the pancreatic lipase. However, most of previous researches just investigated the inhibitive activity of the tested samples against the pancreatic lipase within the range of concentrations instead of the inhibitive mechanism [34,35,36,37]. As shown in the Lineweaver-Burk plots for PL (Figure 2), equations of $Y = 0.0008X + 0.0382$ ($R^2 = 0.9999$), $Y = 0.0014\ X + 0.0668$ ($R^2 = 0.9973$), $Y = 0.002\ X + 0.0969$ ($R^2 = 0.9980$), $Y = 0.0016X + 0.0754$ ($R^2 = 0.9942$), $Y = 0.0012X + 0.0582$ ($R^2 = 0.9931$), $Y = 0.0013\ X + 0.0645$ ($R^2 = 0.9970$), $Y = 0.0018X + 0.086$ ($R^2 = 0.9963$) represent the enzymatic reaction curves for the control, WE, ME, EE, CE, NBE and EAE. The 7 inhibition lines of PL intersect on the x-axis indicated that the mechanism of pancreatic lipase inhibition by WE, ME, EE, CE, NBE and EAE was noncompetitive type. This was similar to the result obtain by Li [3] but diametrically opposed to

Martins [18]. The data showed that the extracts and substrate could bind to pancreatic lipase simultaneously. When both the substrate and the extracts were bound, the enzyme-substrate-inhibitor complex cannot form product, which leaded to a lower activity for enzyme [3].

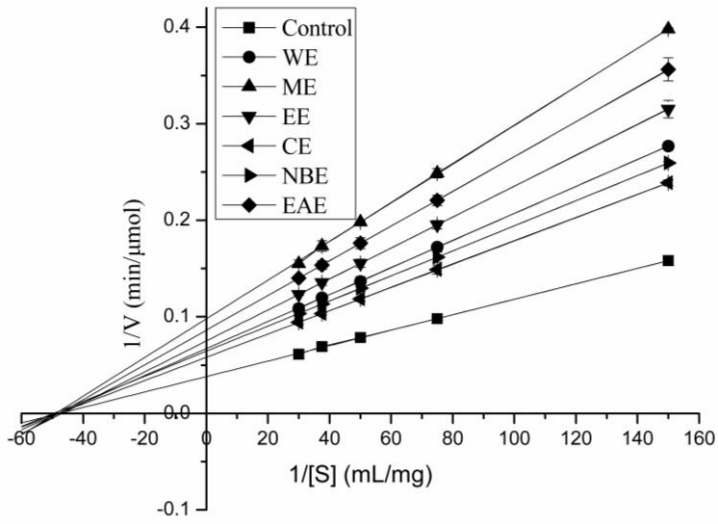


**Figure 2.** Lineweaver-Burk plots of control, water extract (WE), methanol extract (ME), ethanol extract (EE), chloroform extract (CE), N-butanol extract (NBE) and ethyl acetate extract (EAE) for the pancreatic lipase inhibitory activity

**Table 2. $IC_{50}$, $K_i$ and $1/K_i$ values of interaction between pancreatic lipase and different solvents extracts of walnut shell from Zhaotong city in Yunnan province of China**

| Solvent | $IC_{50}$ (μg/mL) | $K_i$ (mg/mL) | $1/K_i$ (mL/mg) |
|---|---|---|---|
| Water | 371.35 ±4.28d | 3.22 ±0.05c | 0.31 ±0.005b |
| Methanol | 308.91 ±4.33c | 2.23 ±0.04b | 0.45 ±0.009c |
| Ethanol | 407.57 ±3.87e | 3.51 ±0.07d | 0.28 ±0.006a |
| Chloroform | 196.50 ±4.13a | 1.72 ±0.01a | 0.58 ±0.004d |
| N-butanol | 247.79 ±3.53b | 2.20 ±0.04b | 0.45 ±0.009c |
| Ethyl acetate | 254.27 ±2.79b | 2.22 ±0.10b | 0.45 ±0.020c |

Data are means of three replicates with standard errors. Values in the same column followed by a different letter are significantly different ($p<0.05$).

The larger of 1/Ki value indicated the stronger inhibition effect on the enzyme activity. It can be seen in Table 2, the sequence of affinity (1/Ki) between pancreatic lipase and the 3 extracts obeying the order: CE > NBE > EAE > ME > WE > EE.

## 3.4. Cell Viability and Triglyceride Assays

To detect the effect of each extract on the viability of 3T3-L1 preadipocytes, MTT was performed after 24 h resulted in on effect on the 3T3-L1 cells viability (Figure 3) which was similar to Lii's results [20]. The result proved that the extracts were non-toxic to 3T3-L1 preadipocytes. The NBE showed the strongest inhibitory effect on triglyceride accumulation of 3T3-L1 preadipocytes (Figure 4). The ME, EE, CE, NBE and EAE had significantly different ($P<0.05$) compared with control. Those data indicated ME, EE, CE, NBE and EAE with strong inhibitory effect on triglyceride accumulation of 3T3-L1 cells. The inhibitory effect of NBE was stronger than 75 μM diallyl trisulfide [20].

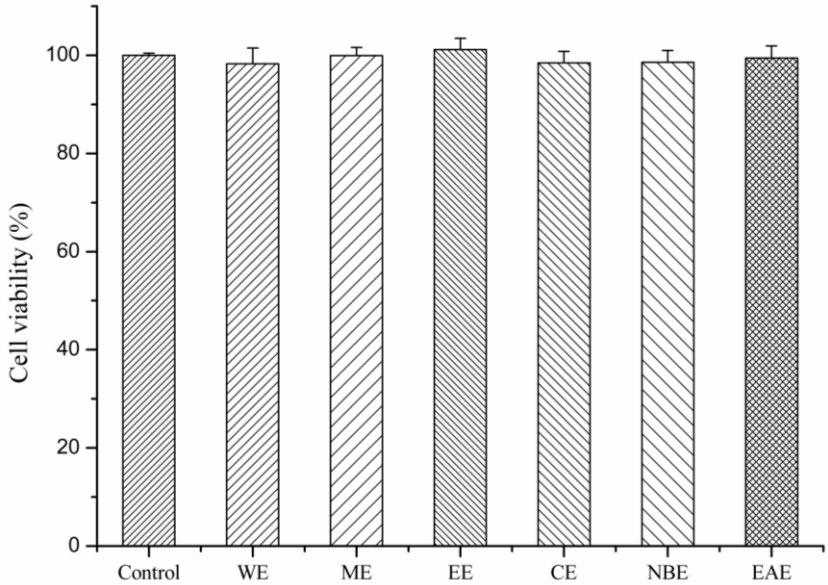


**Figure 3.** Effect of water extract (WE), methanol extract (ME), ethanol extract (EE), chloroform extract (CE), N-butanol extract (NBE) and ethyl acetate extract (EAE) on cell viability of 3T3-L1 preadipocytes cells

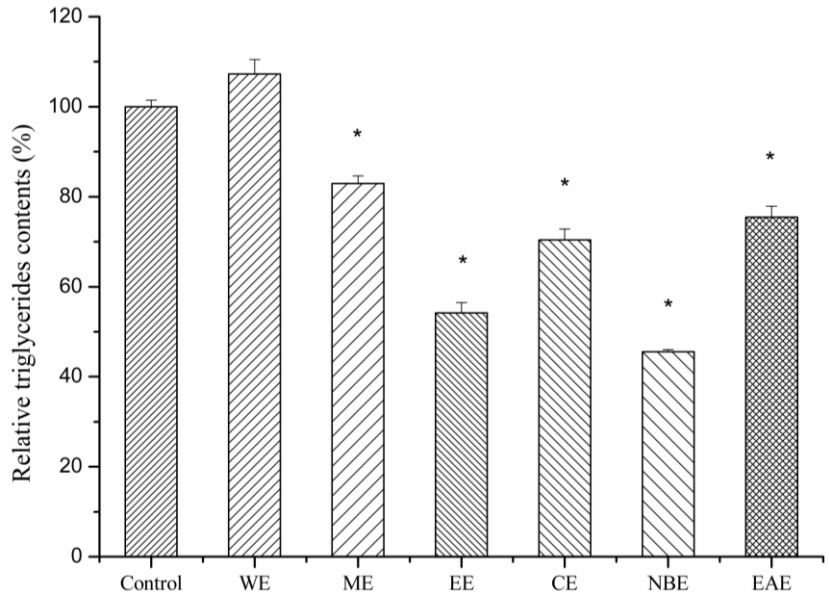


**Figure 4.** Effect of water extract (WE), methanol extract (ME), ethanol extract (EE), chloroform extract (CE), N-butanol extract (NBE) and ethyl acetate extract (EAE) on relative triglycerides contents. * indicated significantly different ($P<0.05$) compared with control

# 4. Conclusions

The inhibitory effect of different solvents (water, chloroform, methanol, ethanol, ethyl acetate and N-butanol) extracts from walnut shell on pancreatic lipase and adipogenesis of 3T3-L1 preadipocytes were demonstrated. The highest EY, TFC and TPC were achieved with N-butanol (4.54%), Ethyl acetate (80.40 mg QEs/g extract) and ethyl acetate (200.40 mg GAE/g extract). The order of inhibitory was ME > EAE > EE > WE > NBE > CE, the inhibition ratios were 82.51%, 73.13%, 70.49%, 69.42%, 65.57% and 60.66%, respectively under the concentration of 500 μg/mL. The $IC_{50}$ values of WE, ME, EE, CE, NBE and EAE were 371.35, 308.91, 407.57, 196.50, 247.79 and 254.27 μg/mL respectively. The inhibitory types of walnut shell extracts were noncompetitive. All the extracts were non-toxic to 3T3-L1 preadipocytes. The ME, EE, CE, NBE and EAE have inhibitory effect on triglyceride accumulation of 3T3-L1 cells. Nevertheless, there is no a clear correlations about the flavonoids and phenols content of extracts and inhibitory effect. This is mainly due to the extracts may be complex mixtures with other active ingredients.

# Acknowledgments

We gratefully acknowledged financial support of the National Natural Science Foundation of China (Grant No. 21466037), Supporting Plan Issue of the Ministry of Science and Technology (2011BAD46B00) and the science and technology major program of Yunnan education bureau (ZD2014009).

# References

[1] Cohen J C, Horton J D, Hobbs H H. Human fatty liver disease: old questions and new insights. *Science*, 2011, 332: 1519-1523.

[2] Bustanji Y, Al-Masri I M, Mohammad M, et al. Pancreatic lipase inhibition activity of trilactone terpenes of *Ginkgo biloba*. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2011, 26: 453-459.

[3] Li Y Q, Yang P, Gao F, et al. Probing the interaction between 3 flavonoids and pancreatic lipase by methods of fluorescence spectroscopy and enzymatic kinetics. *European Food Research and Technology*, 2011, 233: 63-69.

[4] Garza A L, Milagro F I, Boque N, et al. Natural inhibitors of pancreatic lipase as new players in obesity treatment. *Planta Medica*, 2011, 77, 773-785.

[5] Rubio M A, Gargallo M, Isabel Millán A, et al. Drugs in the treatment of obesity: sibutramine, orlistat and rimonabant. *Public Health Nutrition*, 2007, 10: 1200-1205.

[6] Gholamhoseinian A, Shahouzehi B, Sharififar F. Inhibitory effect of some plant extracts on pancreatic lipase. *International Journal of Pharmacology*, 2010, 6, 18-24.

[7] Kim E S, Oh K H, Kim S B, et al. Articles: inhibitory effects of marine algae extract on adipocyte differentiation and pancreatic lipase activity. *Natural Product Sciences*, 2012, 18: 153-157.

[8] Birari R B, Bhutani K K. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discovery Today*, 2007, 12: 879-889.

[9] Gosmann G, Barlette A G, Dhamer T, et al. Phenolic compounds from mate (*Ilex paraguariensis*) inhibit adipogenesis in 3T3-L1 preadipocytes. *Plant Foods for Human Nutrition*, 2012, 67: 156-161.

[10] An Y, Zhang Y, Li C, et al. Inhibitory effects of flavonoids from *Abelmoschus manihot* flowers on triglyceride accumulation in 3T3-L1 adipocytes. *Fitoterapia*, 2011, 82: 595-600.

[11] Srinivasan A, Viraraghavan T. Removal of oil by walnut shell media. *Bioresource Technology*, 2008, 99: 8217-8220.

[12] Pirayesh H, Khazaeian A, Tabarsa T. The potential for using walnut (*Juglans regia* L.) shell as a raw material for wood-based particleboard manufacturing. *Composites Part B: Engineering*, 2012, 43: 3276-3280.

[13] Akbari V, Jamei R, Heidari R, et al. Antiradical activity of different parts of Walnut (*Juglans regia* L.) fruit as a function of genotype. *Food Chemistry*, 2012, 135: 2404-2410.

[14] Fernández-Agulló A, Pereira E, Freire M S, et al. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Industrial Crops and Products*, 2013, 42: 126-132.

[15] Li X, Chen W, Chen D. Protective effect against hydroxyl-induced DNA damage and antioxidant activity of *Radix Glycyrrhizae* (Liquorice Root). *Advanced Pharmaceutical Bulletin*, 2013, 3, 167-173.

[16] Ghasemzadeh A, Jaafar H Z E, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, 2010, 15: 4324-4333.

[17] Zhang Z, He F, Zhuo R. Immobilized lipase on porous silica particles: Preparation and application for biodegradable polymer syntheses in ionic liquid at higher temperature. *Journal of Molecular Catalysis B: Enzymatic*, 2013, 94: 129-135.

[18] Martins F, Noso T M, Porto V B, et al. Mat é tea inhibits in vitro pancreatic lipase activity and has hypolipidemic effect on high-fat diet-induced obese mice. *Obesity*, 2010, 18: 42-47.

[19] Vaidya H, Goyal R K, Cheema S K. Anti-diabetic activity of swertiamarin is due to an active metabolite, gentianine, that upregulates PPAR-g gene expression in 3T3-L1 cells. *Cell*, 2012, 40: 60.

[20] Lii C K, Huang C Y, Chen H W, et al. Diallyl trisulfide suppresses the adipogenesis of 3T3-L1 preadipocytes through ERK activation. *Food and Chemical Toxicology*, 2012, 50: 478-484.

[21] Li X, Ycaza J, Blumberg B. The environmental obesogen tributyltin chloride acts via peroxisome proliferator activated receptor gamma to induce adipogenesis in murine 3T3-L1 preadipocytes. *The Journal of Steroid Biochemistry and Molecular Biology*, 2011, 127: 9-15.

[22] Jaiswal A K, Abu-Ghannam N, Gupta S. A comparative study on the polyphenolic content, antibacterial activity and antioxidant capacity of different solvent extracts of *Brassica oleracea* vegetables. *International Journal of Food Science & Technology*, 2012, 47: 223-231.

[23] Ghasemzadeh A, Jaafar H Z E, Rahmat A. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. *Journal of Medicinal Plants Research*, 2011, 5: 1147-1154.

[24] Jaiswal A K, Rajauria G, Abu-Ghannam N, et al. Effect of different solvents on polyphenolic content, antioxidant capacity and antibacterial activity of Irish York cabbage. *Journal of Food Biochemistry*, 2012, 36: 344-358.

[25] Fern ández-Agull ó A, Pereira E, Freire M S, et al. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Industrial Crops and Products*, 2013, 42: 126-132.

[26] Al-Farsi M A, Lee C Y. Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*, 2008, 108: 977-985.

[27] Bucić-Kojić A, Planinić M, Tomas S, et al. Study of solid–liquid extraction kinetics of total polyphenols from grape seeds. *Journal of Food Engineering*, 2007, 81: 236-242.

[28] Sahreen S, Khan M R, Khan R A. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food chemistry*, 2010, 122: 1205-1211.

[29] Chaleshtori R S, Chaleshtori F S, Rafieian M. Biological characterization of Iranian walnut (*Juglans regia*) leaves. *Turkish Journal of Biology*, 2011, 35: 635-639.

[30] Liu S, Li D, Huang B, et al. Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves. *Journal of Ethnopharmacology*, 2013, 149: 263-269.

[31] Gondoin A, Grussu D, Stewart D, et al. White and green tea polyphenols inhibit pancreatic lipase in vitro. *Food Research International*, 2010, 43: 1537-1544.

[32] Marrelli M, Loizzo M R, Nicoletti M, et al. Inhibition of key enzymes linked to obesity by preparations from Mediterranean dietary plants: effects on α-amylase and pancreatic lipase activities. *Plant Foods for Human Nutrition*, 2013, 68: 340-346.

[33] You Q, Chen F, Wang X, et al. Inhibitory effects of muscadine anthocyanins on α-glucosidase and pancreatic lipase activities. *Journal of Agricultural and Food Chemistry*, 2011, 59: 9506-9511.

[34] Zheng C D, Duan Y Q, Gao J M, et al. Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs. *Journal of the Chinese Medical Association*, 2010, 73: 319-324.

[35] McDougall G J, Kulkarni N N, Stewart D. Berry polyphenols inhibit pancreatic lipase activity in vitro. *Food Chemistry*, 2009, 115: 193-199.

[36] Nakai M, Fukui Y, Asami S, et al. Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *Journal of Agricultural and Food Chemistry*, 2005, 53: 4593-4598.

[37] Jeong J Y, Jo Y H, Lee K Y, et al. Optimization of pancreatic lipase inhibition by *Cudrania tricuspidata* fruits using response surface methodology. *Bioorganic & Medicinal Chemistry Letters*, 2014, 24: 2329-2333.