CUDRANIA TRICUSPIDATA LEAVES EXTRACT AND ITS INHIBITORY ACTIVITY AGAINST HFD-INDUCED OBESITY IN ZEBRAFISH (DANIO RERIO)

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Received Date: 11/8/2023; Revised Date: 06/10/2023; Accepted for Publication: 30/10/2023

ABSTRACT

The research on screening active ingredients from natural resources that effectively for the treatment of metabolic disorders such as obesity and diabetes has received much attention recently. In this study, the compounds extracted from *Cudrania tricuspidata* (CT) leaves were analyzed through FT-IR, HPLC and GC-MS methods. The results showed that 50% ethanol extract (EtOH50) from CT leaves contains several bio-active substances such as catechins, quercetin, gallic acid, etc. In addition, to evaluate the obesity-preventing activity of the extract, an obesity model in adult zebrafish by high-fat diet was carried out. As the results, EtOH50 extract of CT leaves has good efficacy in preventing weight gain, regulating BMI and hyperglycemia in zebrafish. At the same time, based on qRT-PCR results have confirmed that the anti-obesity effect of CT leaves extract via the modulation of several biomarkers related to lipid metabolism, energy expenditure, cytokines genes. Taken together, these data suggest that *Cudrania tricuspidata* (CT) leaves can be developed as a treatment for obesity.

Keywords: *Anti-obesity, Cudrania tricuspidata, HFD-induced obesity, phytochemicals, zebrafish.*

1. INTRODUCTION

Among metabolic disorder syndrome, obesity and diabetes are considered as one of the health problems worldwide. Obesity remains a major risk factor for the development of chronic diseases such as insulin resistance, type 2 diabetes mellitus, and cardiovascular disease (Sato and Mukai, 2020). Obesity is characterized by expansion of adipose tissue resulting in an increase in size and number of adipocytes. To develop therapy for controlling obesity, the regulation of adipogenesis, a complex process of differentiation of pre-adipocytes to adipocytes, is crucial. Adipogenesis is controlled by expression of several adipogenetic transcription factors and genes (Huang *et al.*, 2014; Hirata *et al.*, 2011). During adipogenesis, both transcription factors enhance each other's expression, and activate the expression of lipid metabolism related proteins, such as fatty acid synthetase (*FAS*), and *Leptin*. Moreover, adenosine 5'-monophosphate-activated protein kinase (*AMPK*), a metabolic master switch between anabolism and catabolism, regulates the activities including cholesterol formation, lipogenesis, and lipolysis (Hardie, 2011; Dong *et al.*, 2014; Ahn *et al.*, 2008; Blagih *et al.*, 2015). Although several methods have become an important treatment for metabolic diseases, there has been no current medication that can efficiently prevent obesity.

The zebrafish model (*Danio rerio*), a fascinating subject for biomedical research in recent years, has been widely used to effectively assess safety and bio-ability. In the recently results have suggested that zebrafish can serve as a suitable animal model in research into obesity induced by a HFD (Meguro, Hasumura and Hase, 2015a; Tran *et al*., 2019; Oka *et al*., 2010; Meguro, Hasumura and Hase, 2015b; Landgraf *et al*., 2017). One of the important advantages is that they share a significant amount of genetic characteristics with humans and some zebrafish organ systems are similar to those in humans (Oka *et al*., 2010). Up to 2013, the zebrafish genome was fully sequenced, which is an important basis for understanding and clarifying biological mechanisms (Howe *et al*., 2013).

Recent reports have indicated that the numerous biological activities of phenolic compounds from natural plants. Currently, the search for natural active ingredients with antioxidant, anti-inflammatory or obesity and diabetes treatment support has received much attention. *Cudrania tricuspidata* (CT) Bureau (*Moraceae*) is cudrang, mandarin melon berry and silkworm thron, which has been used as a traditional Chinese medicine (Jeong, Lee and Kim, 2009). Several functions of bioactive compounds from CT plant have been studied. Phytochemicals isolated from the root barks of *Cudrania tricuspidata,* which exhibited a significant hepatoprotective effect (Tian *et al.*, 2005). Glycoprotein isolated from *Cudrania*

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tricuspidata was shown to be an effective anti-inflammatory agent (Shim and Lim, 2009). CT leaves extract has high antioxidant activity and protects pork patties from lipid oxidation and extends shelf-life (Van Cuong and Chin, 2018). On the other hand, research on practical applicability is still very limited, so this is a challenge but also a great opportunity for further studies. Therefore, in the present study, zebrafish were used to investigate how the supplementation of CT leaves extract regulates immune-metabolic pathways in HFD-induced obese zebrafish. The study focused on the lipid metabolism-related genes such as *FAS*, *Leptin*, and the energy metabolism-related genes such as *AMPKα*, *PGC-1α* as well as several cytokine-related genes such as *IL-1β*, *IL-6*, and *TNF-α*, and the ER-related gene *HSPA5* in order try to understand the underlying mechanisms.

2. MATERIALS AND METHODS

2.1. Preparation of CT leaves extract

Fresh CT leaves were obtained from the local market of Gwangju City, South Korea. The leaves were oven-dried at 50°C until the weight was constant for about 72 hr. After drying, they were well ground with a grinder (Hanil Ultra-Power Mixer 3.2L-650W, Gwangju, Korea), then were sieved using a testing sieve 200 μm (Chung Gye Sang Gong Sa, Seoul, Korea). The finely dried powder then was extracted using ethanol 50% (v/v, EtOH50) at a ratio of 1:20 (w/v, 5g:100ml as an example) in combination with ultrasonic assistance at a frequency of 40 kHz with 300 W of generation power (Ultrasonic JAC 2010, 330W, Korea) at 30°C for 30 mins. The extraction was kept in the deep freezer at -70°C prior to lyophilization at -55°C (Ilshin freeze dryer, Korea) for about 3 days until it was completely dried.

2.2. FT-IR spectroscopy

The dried powder of the extract by EtOH50 from CT was subjected to FT-IR analysis. The FT-IR spectrum recorded the absorption by wavelength ranging from 4000 to 380 cm^{-1} in the Spotlight 400 FT-IR, Perkin Elmer systems.

2.3. HPLC analysis

The HPLC analysis of major phenolic and flavonoid components from CT leaves extract (by EtOH50) was operated by using HPLC systems (LC-10Avp Shimadzu Co., JAPAN) with C18 column (Shimadzu, Shim-pack CLC-ODS (5 μm, 250 mm × 4.6 mm). The mobile phase consisted of acetonitrile : acetic acid : methanol : water (113:5:20:862, v/v/v/v). The HPLC conditions were as follows: The flow rate (0.5 mL/min), column temperature (30°C), the sample injection volume (10 μL), and the UV detection wavelength (280 nm). An aliquot of 10 μL solution (extract sample) was injected for HPLC analysis after filtration with a 0.22 μm membrane filter. The absorbance was read by the UV-Vis detector with wavelength set at 280 nm. Identification of the phenolic and flavonoid compounds was carried out by comparing their retention times to those of standards (gallic acid, catechin, vanillic acid, rutin, and quercetin). Gallic acid, catechin, vanillic acid, rutin, and quercetin were purchased from Sigma-Aldrich (Merck Sigma Chemical Co., St. Louis City, MO, USA). All other chemicals (acetonitrile, methanol, acetic acid, deionized water) were of analytical grade.

2.4. GC-MS analysis of phytochemical components

The phytochemical compounds of EtOH50 were analyzed. GC-MS study was carried out by using GCMS-QP2010 systems (Shimadzu Co., Kyoto, Japan). The analytical conditions were as follows: Injection temperature at 250°C; Column temperature: 80°C (2 min) – (15°C/min) - 320°C (20 min); Injection mode: Split; Carrier gas: He (Constant Linear Velocity); Linear velocity: 36.8 cm/sec; Split ratio: 25:1; Injection volume: 1 μL. Molecular ions (mass range) were monitored for identification which was set at 50-500 m/z. For identification of phytochemical composition, the database of National Institute Standard and Technology MS library (NIST05) and Wiley Spectral library (WILEY07) were used to compare.

2.5. Zebrafish care and experimental design

Adult zebrafish (*Danio rerio*) 200 – 300 mg in body weight at around 3 months old were purchased from a local aquarium shop in Gwangju, South Korea and kept under a 14-h light/10-h dark cycle (turn on at 8 am and turn off at 10 pm) at a constant temperature of 28±0.5°C for a week prior to the experiment. After acclimatization time, 25 fish per group were randomly assigned into three experimental groups; Normal feeding diet: NFD, High fat feeding diet: HFD and High fat feeding diet+CT leaves extract: HFD+CT) and maintained in a 4-L aquarium tank. During the experiment period, fish in all groups were fed twice daily (at 9:00 am and 6:00 pm, for 10 weeks). Commercial fish diet (Tetra Bits Complete fish food consists

of 9% fat, 17% carbohydrate and 59% protein) was purchased from the local market (Gwangju, South Korea). Fish diets were prepared based on commercial feed diet, in fact, the high-fat diet formulation (HFD) was added 25% of lard (back fat) to a commercial diet, while the HFD+CT, CT leaves extract was added at the concentration of 2.0% to the HFD diet. All experiments related to zebrafish were raised according to the Zebrafish Book, Ed. 4, (2000) (visit at https://zfin.org/zf_info/zfbook/zfbk.html).

2.6. Body weight and fasting blood glucose measurements

During the 10-week feeding period, five zebrafish were randomly collected from each group on day 0 and then 5 weeks and 10 weeks to determine the body weight. At the same time, the body length (mm) was recorded and the BMI (mg/mm2) was calculated as described previously (Oka *et al*., 2010). In addition, during 10 weeks of feeding period, blood samples were collected at each time point (day 0, after 5 weeks, and after 10 weeks) to determine the fasting blood glucose levels. Firstly, fish were randomly selected (3 zebrafish/group), then transferred into a clean separate tank and kept overnight for 12 hr without feeding. Blood samples from the individual zebrafish were collected from the dorsal artery and immediately measured using a glucometer (CareSens II Plus+, Informa Life Sciences) with twice measurement of a zebrafish (Tran *et al*., 2019).

2.7. RNA extraction, cDNA synthesis, and quantitative real-time RT-PCR

Total RNA was extracted from the whole body of adult zebrafish using RNAiso Plus reagent (TaKaRa Bio, Shiga, Japan) according to the manufacturer's instructions. After RNA isolation, total RNA concentration was measured using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, USA), and equalized to all the groups. The equalized RNA was used for cDNA synthesis using a TaKaRa PCR Thermal Cycler Dice Real-Time System as the following conditions: 63°C for 10 min, 37°C for 60 min and 95°C for 5 min. Briefly, each 10 µL aliquot of equalized RNA was transcribed into cDNA using 1 µL random primer, 2 µL dNTP mixture, 0.5 µL recombinant RNase inhibitor (TaKaRa Bio), 1 µL MMLV RT, 4 µL 5X MMLV RT buffer, and 2 µL 5X DTT (Beams Biotechnology, Seongnam, Korea). Finally, each 2 µL of those was used as a template for real-time qPCR. Real-time qPCR was carried out in TaKaRa Thermal Cycler Dice Real-Time System using SYBR Premix Ex Taq™ kit (TaKaRaBio). In the current experiment, we analyzed the several function genes, which target various biological indexes including inflammation and immune-related genes using gene-specific primers (Table 1). Quantitative real-time PCR was performed in a Thermal Cycler DiceReal-Time System (Takara Bio) using a 21 µL reaction mixture containing 10µL SYBR Green Master Kit (TaKaRa Bio, Japan), 1 µL for each Forward primer and Reverse primer, 2 µL template cDNA, and 7 µLRNAase free water. The amplification conditions for the real-time PCR were as follows: 30-second hold at 95°C, 45 cycles of denaturation for 5 seconds each at 95°C, 10 seconds at 55°C, and 20 seconds at 72°C and 1 cycle of dissociation at 95°C for 15 seconds, 60°C for 30 seconds and 95°C for 15 seconds. Beta-actin was used as the reference gene for normalization. The mRNA expression levels of target genes were determined by the $2^{-\Delta\Delta Ct}$ method (ΔCt = Ct was for a target gene, Ct for reference gene; ΔΔCt = ΔCt was for treat group – ΔCt for the control group). All the quantitative real-time PCR reactions were run in triplicate.

Table 1. Details of the primer sequence used for qRT-PCR

Primer	Sequence (5' – 3')	Description
FAS	Forward: GAGAAAGCTTGCCAAACAGG Reverse: GAGGGTCTTGCAGGAGACAG	plays important roles in lipogenesis genes; fatty acid synthase
Lepin	Forward: AGCTCTCCGCTCAACCTGTA Reverse: CAGCGGGAATCTCTGGATAA	lipogenesis genes
AMPK-a	Forward: AGTTATCAGCACACCGACAG Reverse: AGTAATCCACCCCTGAGATG	AMPK, AMP-activated protein kinase
PGC-1a	Forward: GGCCCAGCGAGCCAAACCAA Reverse: TGGCTTTGTGAGGAGGCGTGG	The peroxisome proliferator–activated receptor γ coactivators 1α (PGC-1α), a central regulator of metabolism

Primer	Sequence (5' – 3')	Description
IL-1β	Forward: ATCCAAACGGATACGACCAG Reverse: TCGGTGTCTTTCCTGTCCAT	interleukin-1b; as regulatory molecules in the coagulation cascade in obese zebrafish
IL6	Forward: TCAACTTCTCCAGCGTGATG Reverse: TCTTTCCCTCTTTTCCTCCTG	interleukin 6; as regulatory molecules in the coagulation cascade in obese zebrafish
TNF-a	Forward: AGGCAATTTCACTTCCAAGG Reverse: AGGTCTTTGATTCAGAGTTGTATCC	tumor necrosis factor alpha
HSPA5	Forward: AAGAGGCCGAAGAGAAGGAC Reverse: AGCAGCAGAGCCTCGAAATA	endoplasmic reticulum (ER) stress genes
β-actin	Forward: ATGAAGATCCTGACCGAG Reverse: TAGCTCTTCTCCAGGGAG	House-keeping gene, for normalization

2.8. Statistical analysis

Statistical analysis was carried out using SPSS 21.0. Data are expressed as the mean ± standard deviation (SD) and were analyzed by one-way ANOVA with post hoc analysis followed by Tukey's or Duncan's tests. GraphPad Prism 5 (GraphPad, La Jolla, CA, USA) was used to draw figures from qRT-PCR results. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. FT-IR spectrum study of Cudrania tricuspidata (CT) leaves extract

FT-IR analysis was carried out to identify the chemical structure of individual antioxidant components from CT leaf. The extracts of CT leaves using ethanol 50% (EtOH50) were subjected to FT-IR analysis and the functional groups were identified. As shown in Figure 1, the first peak of the extract was around 3252 to 3292 cm^{-1} (3285 cm^{-1}) responded that these compounds contain O-H linkage, which indicate phenolic, alcohol or hydroxyl groups. Other functional groups from the CT leaves extract were detected including 2924.69 cm^{-1} (-CH stretching vibration), 1595.23 cm^{-1} (-NH stretching vibration), 1364.72 cm^{-1} and 1267.76 cm^{-1} (-CH2 stretching), 1039.75 cm^{-1} and 987.0 cm^{-1} (C-C, C-OH, -CH ring and side group vibrations). The results of FT-IR analysis confirmed that the powder of CT leaf extracts contains alcohol, phenol, alkane, alkyne, aromatics, hydrocarbons, and amines. The results in the current study are consistent with our previous study (Thoa and Cuong, 2018). In addition, the results are similar to the finding of Subashini *et al*. (2015) who reported that *Gymnema sylvestre* leaves contained alcohols, phenols, alkanes, alkynes, alkyl halides aldehydes, carboxy acids aromatics, and aromatic amines (Subashini *et al.*, 2015). Sangeetha *et al*. (2014) stated that the presence of aliphatic and aromatic amines and alkenes in *Gymnema sylvestre* might contribute to the antioxidant activity (Sangeetha, Archit and SathiaVelu, 2014). In addition, a previous study by Jabamalairaj *et al*. (2015) on *Citrus grandis* (L.) leaves indicated that the presence of functional groups such as alcohol, alkane, amines, aromatics, aldehydes, phenols, esters and nitro compounds correlated with antimicrobial activity (Anitha Jabamalairaj, Alagar Yadav and Bathrachalam, 2016). Thus, these compounds from EtOH50 extract of CT leaves may possess both antioxidant and antimicrobial activities.

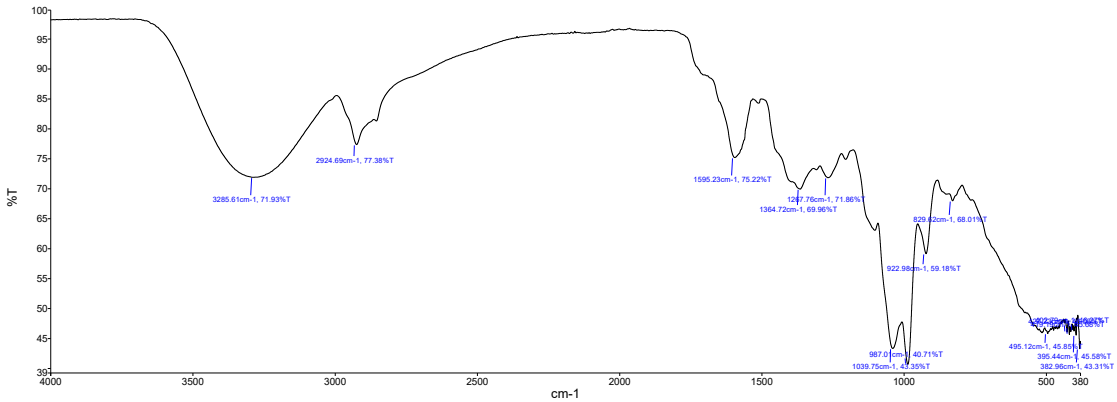

Figure 1. FT-IR spectrum analysis of CT leaves extract using ethanol 50% (EtOH50)

3.2. HPLC analysis of individual components from CT leaves extract

The extract of CT leaves using EtOH50 was then subjected to further analysis by HPLC. As a result, the EtOH50 extract from *C. tricuspidata* leaves contained a variety of bioactive compounds. By comparing with the standards including vanilic acid, two main flavonoids (quercetin, rutin) and two major phenolics (gallic acid, catechin), these compounds were detected and quantified. As shown in Figure 2 and Table 2, the HPLC results indicated that catechin was the predominant polyphenol in *C. tricuspidata* leaf by EtOH50 extract, which contained 6841.31±87.23 mg/100 g in EtOH50 extract. And, then it followed by quercetin, which contains 1216.15±34.87 mg/100 g. The HPLC results provide in detail the information about individual bioactive components from *C. tricuspidata* leaves. Based on the composition result of extracts from *C. tricuspidata* leaves, these compounds (particularly gallic acid, catechin, rutin and quercetin) could be major components to the antioxidant activities of the extract. Flavonoids and phenolic acids are also major factors that contribute to the antioxidant activity and also have many health benefits. Therefore, this study suggested that *C. tricuspidata* leaf would be a potential source of bio-active compounds for further application research.

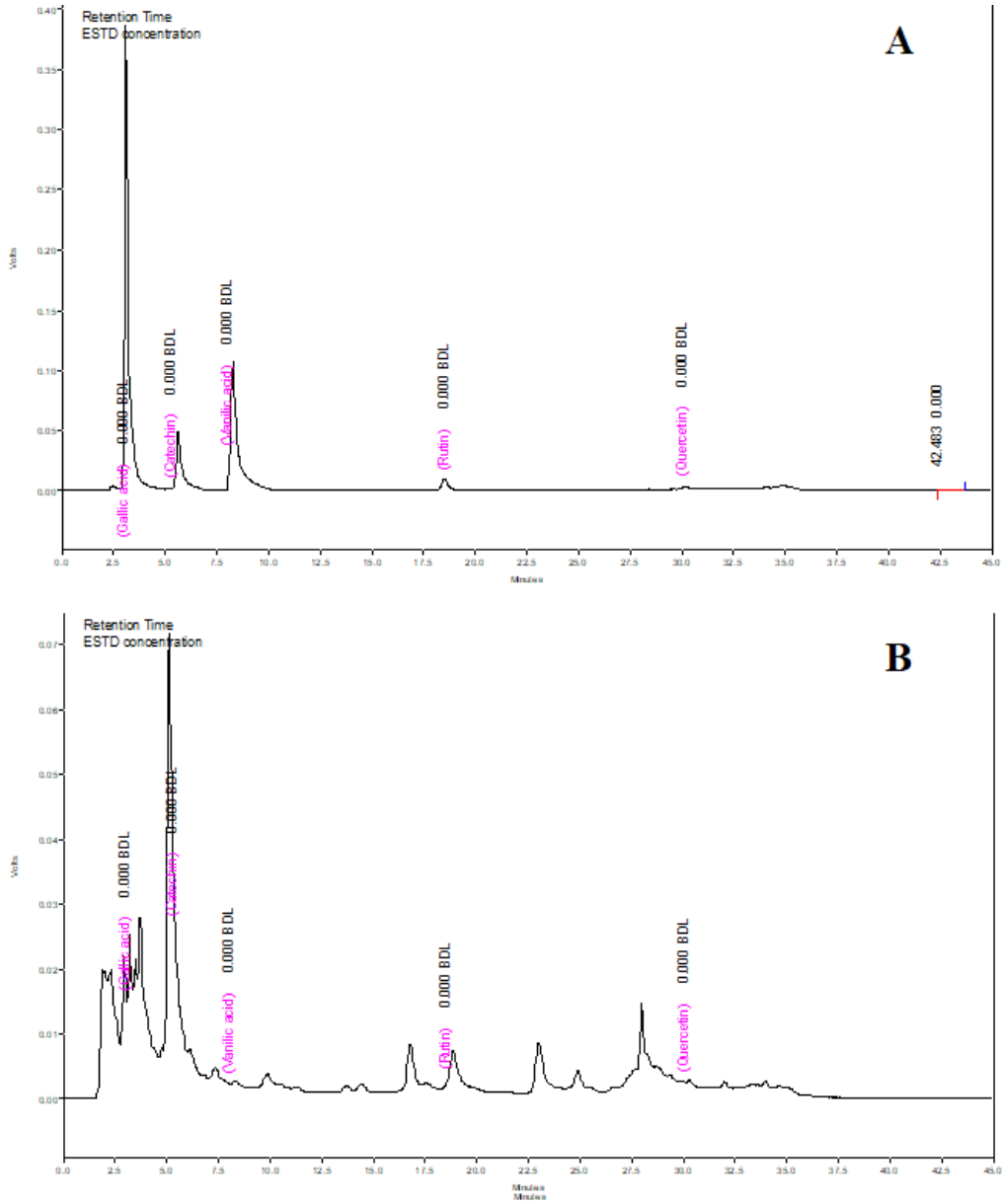

Figure 2. HPLC chromatograms analysis of five polyphenol standards and ethanol 50% (EtOH50) extraction from CT leaves.

Note: A. HPLC of five standards (phenolic and flavonoid compounds); B. HPLC of EtOH50 extract of CT leaves.

Table 2. Individual components of CT leaves by EtOH50 extraction

(Unit: mg/100g DW)

Individual components				
Gallic acid	Catechin	Vanilic acid	Rutin	Quercetin
76.57±5.45	6841.31±87.23	76.45±3.76	55.55±1.98	1216.15±34.87

3.3. GC-MS study of phytochemical components from CT leaves extract

C. tricuspidata has been used as natural medicine from plant resources for a long time, however to our knowledge there are no reports on the phytochemical composition of this leaf as affected by different solvents. In the present study, GC-MS analysis was performed to analyze phytochemical components from C. tricuspidata leaves by EtOH50 extraction. The identified compounds of the C. tricuspidata leaves in 50% ethanolic extract are shown in Figure 3. As the results, there are many compounds detected in the extract. The major components present in EtOH extract includes 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (28.95%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- (13.03%), Methyl (Z)-5,11,14,17-eicosatetraenoate (8.93%), Stigmast-5-en-3-ol, (3.beta.)- (7.09%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (6.17%) (Table 3). There are several authors who studied the correlation between phytochemical constituents and their biological activities. Sugumar and Karthikeyan (2015) have analyzed chemical composition using GC-MS and its antimicrobial activity of essential oil from Eupatorium triplinerve Vahl (Sugumar, Karthikeyan and Gowdhami, 2015). Ahmad et al. (2015) reported the positive effect of phytochemicals from *Mangifera pajang* on human breast cancer, cervical cancer and colon cancer cells (Ahmad et al., 2015). Therefore, the preliminary results of present study provide more details about phytochemical components of *C. tricuspidata* and this is not only for better understanding but also useful for further studies, which could be apply to other area such as pharmaceutical and functional food.

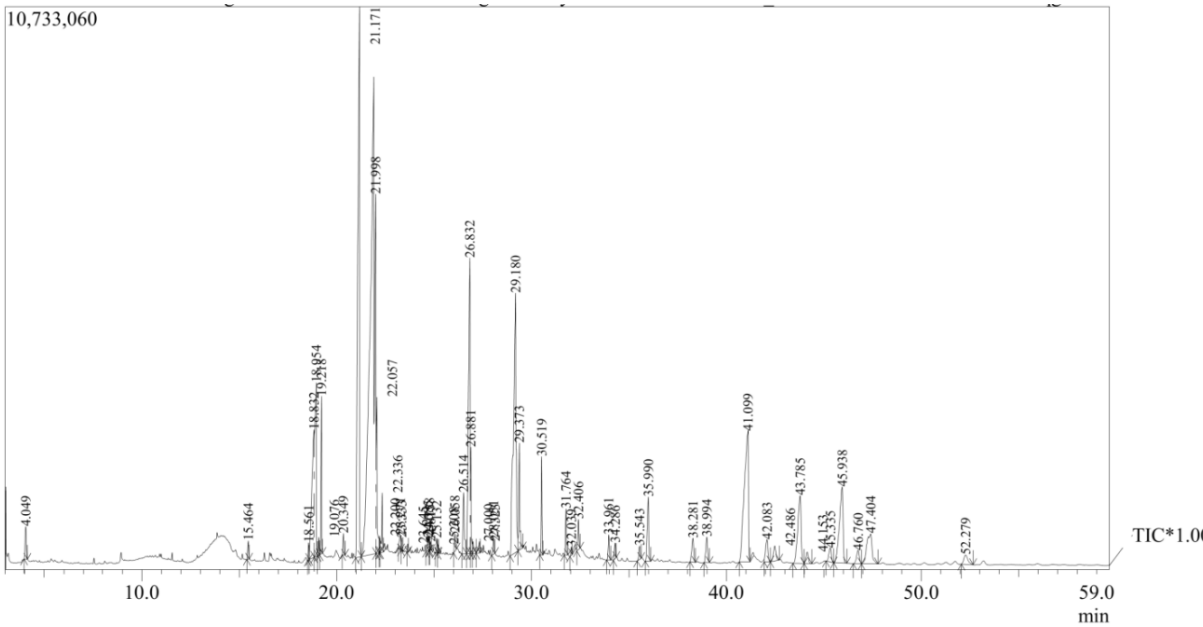

Figure 3. GC-MS analysis of EtOH50 extraction from CT leaves

Table 3. Phytochemical components identified of EtOH50 extraction from CT leaves by GC-MS analysis

EtOH50 extraction from *C. tricuspidata* leaves					
R.T.	**Compounds**	**Peak area (%)**	**R.T.**	**Compounds**	**Peak area (%)**
18.832	Hexadecanoic acid	3.97	26.832	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	6.17
18.954	Hexadecanoic acid	3.76			
19.218	Hexadecanoic acid, ethyl ester	1.48			
21.171	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	13.03	29.180	Methyl (Z)-5,11,14,17-eicosatetraenoate	8.93
21.998	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	28.95	29.373	Octadecanoic acid, 2-hydroxy-1-Vitamin E	1.42
	Octadecanoic acid		35.990	Stigmast-5-en-3-ol,	1.29
	(Z)14-Tricosenyl		41.099	(3.beta.)-	7.09
22.057	formate	1.70		Methyl commate B	
26.514		1.11	43.785	Vitamin E	2.99
			45.938	Methyl commate B	3.66
			47.404		2.09

3.4. CT suppresses HFD-induced obesity in zebrafish

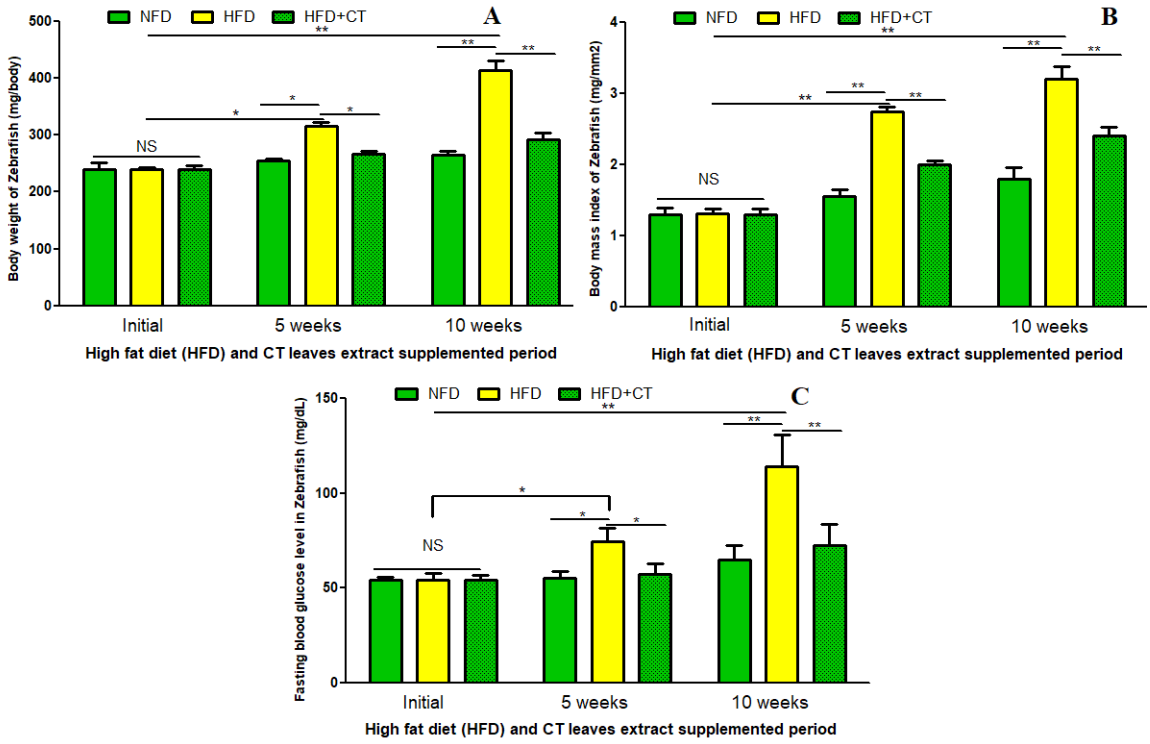

Figure 4. A. Changes in body weight; B. Changes in BMI; C. Changes in fasting blood glucose in zebrafish in normal feeding diet (NFD), high fat diet (HFD) and high fat diet treated CT leaves extract (HFD+CT) groups.

*Note: All experimental zebrafish were randomly assigned to 3 groups (n=25 per group) based on the types of diet. The values were measured at initial (day 0) and every 5 weeks up to 10 weeks of treatment in NFD, HFD, HFD+CT fed groups. Values are average of three zebrafish of each groups, each value is mean ± SD and *p < 0.05, **p < 0.01.*

As expected, after the 10-week experiment, the fish had significantly gained weight in all groups as compared to their initial weights (day 0), indicating normal growth. The HFD group showed a significantly higher increase in body weight than the NFD group, which has started showing a significant

difference after 5 weeks of feeding (Figure 4A). This result also shows similarities with our previous study on creating obesity using zebrafish models (Tran *et al*., 2019). CT leaves extract attenuated weight gain; in the HFD+CT fed-group, mean body weight was not significantly higher than in the NFD group after ten weeks of feeding. Similarly, the BMI was calculated, and the results showed a significant increase in all groups at the end of the experiment, but again, the HFD group showed the highest BMI among the three groups, whereas it did not significantly differ between NFD and HFD+CT (Figure 4B). HFD significantly elevated the fasting blood glucose level (114.2 ± 16.8 mg/dL vs. 65.0 ± 7.7 mg/dL in NFD, $p < 0.01$), whereas CT extract had a significant suppressive effect 72.4 ± 11.2 mg/dL, $p < 0.05$) (Figure 4C). Our data indicated that the zebrafish obesity model was successfully established by ten weeks of HFD. In addition, the present results clearly indicated that CT leaves extract has an anti-obesity effect as it attenuated weight gain and BMI and decreased blood glucose levels in HFD-fed zebrafish. Based on the results of identifying some secondary compounds through HPLC analysis from CT leaves, it is suggested that two main components including catechin and quercetin might act as bioactive compound that prevent weight gain causing obesity in zebrafish. Several authors have mentioned that catechins from natural edible plants are well-known to be effective in reducing the risk of obesity (Ohyama et al., 2011; Kim et al., 2019). Previous study reported that catechins from green tea have activity to prevent obesity through modulation of peroxisome proliferator-activated receptors (Yan, Zhao and Zhao, 2013). Another study also using zebrafish as a model to induce obesity have reported the similarity. In this study, the authors stated that adding green tea extract rich in catechin at the level of 5% to the high- fat diet significantly suppressed body weight, body fat volume (Meguro, Hasumura and Hase, 2015a). Quercetin is the major representative of the flavonoid subclass of flavonols, which is ubiquitously contained within natural plants such as green tea, and vegetables, fruits, including onions and apples (Sato and Mukai, 2020). Several previously authors have reported the beneficial effects of quercetin on obesity and diabetes. Ahn et al. have reported that quercetin has the anti-obesity effect by mediating the AMPK and MAPK signaling pathways (Ahn *et al*., 2008).

3.5. CT leaves extract suppresses lipogenesis and elevates energy expenditure in obese zebrafish

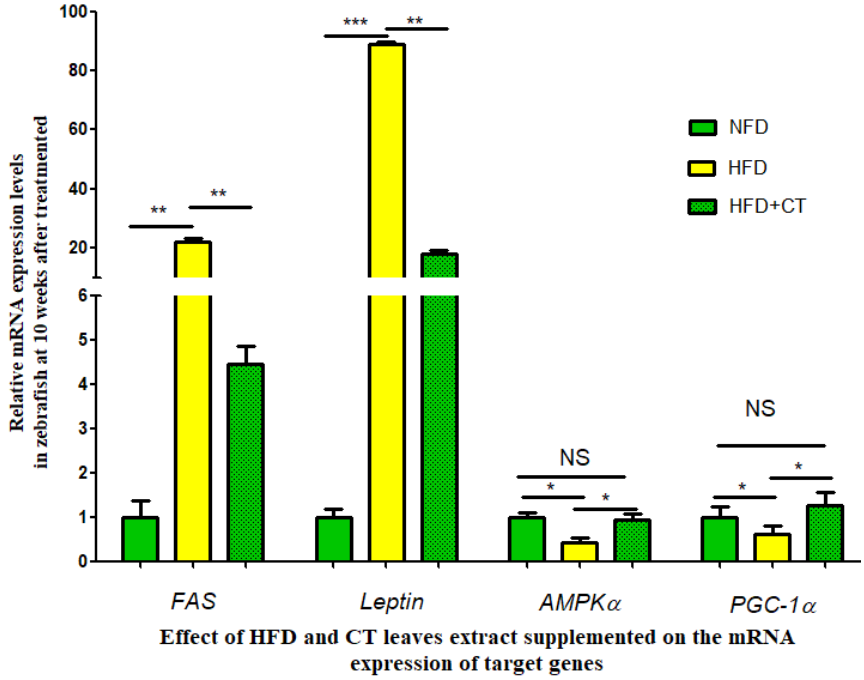

Figure 5. Effect of HFD and CT leaves extract supplemented on the mRNA expression of target genes.

*Note: CT leaves extract modulates mRNA expression of the lipid metabolism-related genes: FAS, Leptin, the energy metabolism-related genes AMPKα, PGC-1α. Results are means ± SD of 3–4 experiments. *p < 0.05, **p < 0.01, and ***p < 0.001.*

To investigate the effects of HFD and the potentially regulatory effect of CT leaves extract on lipid metabolism, we evaluated the expression of genes encoding leptin (*Leptin*), fatty acid synthase (*FAS*). As the results are shown in Figure 5, HFD highly induced expression of *FAS* and *Leptin*,

transcript levels in zebrafish after the ten-week feeding period, indicating it induced lipogenesis. CT leaves extract supplementation significantly reversed HFD-induced lipogenesis-related gene expression. This result is completely consistent with our deprivation experiment on using an obese zebrafish model (Tran *et al*., 2019). Moreover, our findings in the current study showing the similarity of previous research, which mentioned that overfeeding strongly induced FAS expression in adult zebrafish (Landgraf *et al*., 2017). Inhibiting *FAS* transcription to play an important role in preventing obesity has been studied and discussed in many previous reports (Chakravarthy *et al*., 2009; Gupta *et al*., 2013). In agreement with our results, Simonds *et al*. reported that *Leptin* plays an important role in the increase in blood pressure and body weight associated with obesity in mice (Simonds *et al*., 2014). Several authors have also discussed about the relationship between leptin resistance and diet-induced obesity (Lin *et al*., 2000; Lee *et al*., 2016); Sáinz *et al*., 2015). In humans, leptin mRNA concentrations in adipose tissue and serum positively correlate with the amount of fat mass, and fasting or weight loss lowers leptin levels (Van Der Klaauw and Farooqi, 2015).

In addition, we evaluated whether CT leaves extract enhances whole-body energy expenditure in HFD, by measuring the expression of energy metabolism-related genes encoding AMP-activated protein kinase α (*AMPKα*), peroxisome proliferator-activated receptor gamma coactivator 1 α (*PGC-1α*) using qRT-PCR. As a result, HFD dramatically reduced the expression of *AMPKα, PGC-1α*, the energy metabolism-related genes. Interestingly, CT leaves extract significantly elevated the expression of these genes to levels observed in the NFD group. Adenosine 5'-monophosphate-activated protein kinase (*AMPK*) plays a major role in glucose and lipid metabolism and to control metabolic related disorders, including obesity. Moreover, *AMPK*, a metabolic master switch between anabolism and catabolism, regulates the activities including cholesterol formation, lipogenesis and lipolysis (Ahn *et al*., 2008; Wu *et al*., 2018; Murase *et al*., 2009; Song *et al*., 2015). The activation of *AMPK* switches off ATP-consuming anabolic pathways such as gluconeogenesis, fatty acid synthesis, protein synthesis, and cholesterol synthesis, and switches on ATP-generating catabolic pathways such as β-oxidation, glucose uptake, and glycolysis. PGC-1α is known to suppress ROS, activate glycolysis, and play a role in fatty acid oxidation and energy metabolism. Therefore, by elevating energy expenditure is one of the best approaches to fight obesity (Meguro, Hasumura and Hase, 2015a). As *AMPKα*, *PGC-1α*, are considered vital nutrients and energy sensors, it is highly likely that the supplementation of CT leaves extract promotes energy expenditure in HFD-fed zebrafish by elevating their activities. Mainly, the *AMPK* and PGC-1α were predicted to be activated in HFD+CT group compared to HFD group leading to up-regulation of fatty acid oxidation and down-regulation of fatty acid synthesis.

3.6. Changes in inflammation and the immune system by HFD and CT leaves extract supplemented

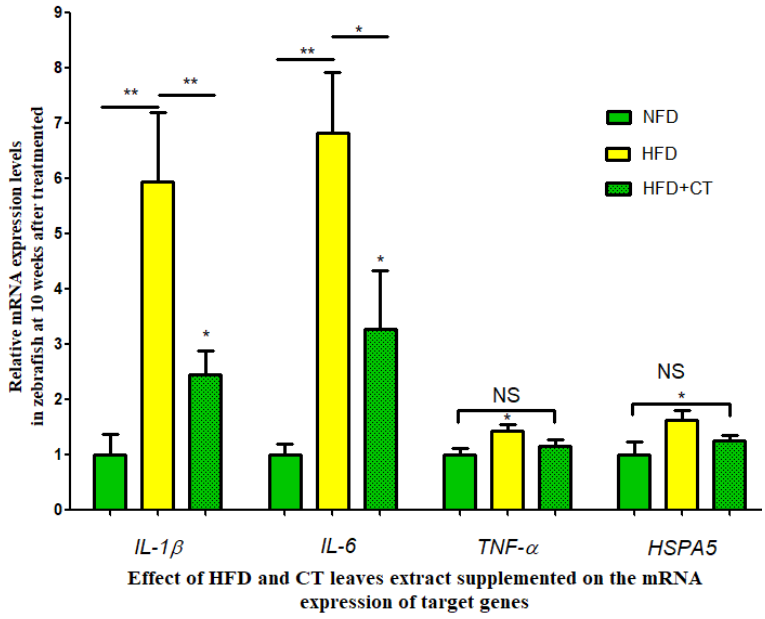

Figure 6. Effect of HFD and CT leaves extract supplemented on the mRNA expression of target genes in zebrafish.

*Note: CT leaves extract modulates mRNA expression of the inflammatory cytokines related genes IL-1β, IL-6, and TNF-α, and the ER-related gene HSPA5. Results are means ± SD of 3–4 experiments. *p < 0.05, **p < 0.01.*

To evaluate the potentially inhibitory effect of CT leaves extract on HFD-induced inflammation on adipokine production in zebrafish, we evaluated expression levels of inflammatory genes encoding interleukins (*IL-1β, IL-6*), and tumor necrosis factor α (*TNF-α*) as well as the endoplasmic reticulum chaperone (*HSPA5*, also is called heat shock protein 5) in all treatment groups after 10-week treatment. The data are shown in Figure 6, mRNA levels of all tested pro-inflammatory genes were much increased in the HFD group compared to the NFD group. Meanwhile, CT leaves extract supplemented treatment significantly inhibited the expression of these pro-inflammatory cytokines and ER chaperone. The results of our study are supported by a previous study, which reported that a compound from CT leaves suppressed tumor necrosis factor-α (*TNF-α*) and interleukin-1β (*IL-1β*) production (Jeong, Lee and Kim, 2009). As shown in the current findings, suggested that HFD induces inflammation, which may further induce obesity in zebrafish. This result is also consistent with our team's previous study of HFD-induced obesity and high glucose-induced hyperglycemia in adult zebrafish (Tran et al., 2019; Cuong and Thoa, 2020). The heat shock protein 5 (HSPA5) was considered as a biomarker since ER stress markers have been highly responsive to a variety of stresses, specifically promote metabolic disease progression (Laybutt *et al.*, 2007; Shan *et al*., 2017; Sharma *et al*., 2008). Recently, Ajoolabady et al. (2022) have summarized the role of ER stress and the ER stress response in the development of obesity. Authors suggested that ER stress is possible to drive adiposity by decreasing energy expenditure, making ER stress a promptly therapeutic target for the prevention of obesity (Ajoolabady *et al*., 2022). Our findings reinforce the strong basis for the association between ER stress and obesity, opening the prospect of obesity treatment based on modulating ER stress markers.

4. CONCLUSION

In the present work, for the first time, the anti-adipogenic and anti-obesity effect of CT leaves extract was observed in zebrafish experiment. CT leaves extract selectively suppressed fatty acid synthesis and activated energy catabolism in HFD-induced obese zebrafish. The anti-adipogenetic effect could be due to down-regulation of different transcription factors such as *FAS*, *Leptin* or activating of *AMPKα*, *PGC-1α*. Moreover, the heat shock protein 5 (HSPA5) was considered as an important biomarker, promising as a therapeutic target for the prevention of obesity. In conclusion, CT leaves extract has great potential to reduce adipogenesis in HFD-induced obesity in zebrafish by regulating multiple genes and proteins. It suggested that CT leaves can be developed as a therapeutic agent for obesity. Therefore, future work will be necessary to understand the underlying mechanisms involved with a view to identifying novel therapeutic strategies for obesity prevention.

CHIẾT XUẤT LÁ CUDRANIA TRICUSPIDATA VÀ KHẢ NĂNG ỨC CHẾ CỦA NÓ NGĂN NGỪA BỆNH BÉO PHÌ DO CHẾ ĐỘ ĂN NHIỀU CHẤT BÉO GÂY RA Ở CÁ NGỰA VẰN (DANIO RERIO)

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Ngày nhận bài: 11/8/2023; Ngày phản biện thông qua: 06/10/2023; Ngày duyệt đăng: 30/10/2023

TÓM TẮT

Nghiên cứu sàng lọc các hoạt chất từ tự nhiên có hiệu quả trong điều trị các rối loạn chuyển hóa như béo phì và tiểu đường đã nhận được nhiều sự quan tâm trong thời gian gần đây. Trong nghiên cứu này, chúng tôi đã phân tích một số hợp chất chiết xuất cồn 50% (EtOH50) từ lá *Cudrania tricuspidata* (CT) thông qua các phương pháp FT-IR, HPLC và GC-MS. Kết quả cho thấy, chiết xuất EtOH50 từ lá CT có chứa một số hoạt chất sinh học như catechin, quercetin, gallic acid, ... Ngoài ra, chúng tôi đã tiến hành đánh giá hoạt tính ngăn ngừa béo phì của chiết xuất bằng cách sử dụng mô hình gây béo phì ở cá ngựa vằn trưởng thành bằng chế độ ăn có hàm lượng chất béo cao. Kết quả thực nghiệm cho thấy, chiết xuất EtOH50 của lá CT có hiệu quả tốt trong việc ngăn ngừa tăng cân, điều chỉnh chỉ số trọng lượng cơ thể và có tác dụng ngăn ngừa sự tăng đường huyết ở cá ngựa vằn. Đồng thời, dựa trên kết quả phân tích qRT-PCR đã khẳng định tác dụng chống béo phì của chiết xuất lá CT thông qua việc điều tiết một số chỉ thị sinh học (gene) liên quan đến quá trình chuyển hóa lipid, tiêu hao năng lượng, và các gene cytokine. Dựa trên những dữ liệu tại nghiên cứu này cho thấy lá *Cudrania tricuspidata* (CT) có thể được phát triển như một phương pháp điều trị béo phì tiềm năng.

Từ khóa: *Chống béo phì, Cudrania tricuspidata, chế độ ăn nhiều chất béo, hợp chất thứ cấp, cá ngựa vằn.*

REFERENCES

Ahmad, S. *et al.* (2015) 'Phytochemicals from Mangifera pajang Kosterm and their biological activities', *BMC Complementary and Alternative Medicine*, 15(1), p. 83. doi: 10.1186/s12906-015-0594-7.

Ahn, J. *et al.* (2008) 'The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways', *Biochemical and Biophysical Research Communications*, 373(4), pp. 545–549. doi: 10.1016/j.bbrc.2008.06.077.

Ajoolabady, A. *et al.* (2022) 'ER stress in obesity pathogenesis and management', *Trends in Pharmacological Sciences*. Elsevier Ltd, 43(2), pp. 97–109. doi: 10.1016/j.tips.2021.11.011.

Anitha Jabamalairaj, Alagar Yadav, S. and Bathrachalam, C. (2016) 'Characterization of Functional Group Analysis and Antimicrobial Activity of Leaf Extracts of Citrus grandis (L.) against Clinical Pathogens', *Indo American Journal of Pharmaceutical Research*, 5(5), pp. 1642–1648.

Blagih, J. *et al.* (2015) 'The Energy Sensor AMPK Regulates T Cell Metabolic Adaptation and Effector Responses InVivo', *Immunity*, 42(1), pp. 41–54. doi: 10.1016/j.immuni.2014.12.030.

Chakravarthy, M. V. *et al.* (2009) 'Inactivation of hypothalamic FAS protects mice from diet-induced obesity and inflammation', *Journal of Lipid Research*. © 2009 ASBMB. Currently published by Elsevier Inc; originally published by American Society for Biochemistry and Molecular Biology, 50(4), pp. 630–640. doi: 10.1194/jlr.M800379-JLR200.

Van Cuong, T. and Chin, K. B. (2018) 'Evaluation of Cudrania tricuspidata Leaves on Antioxidant Activities and Physicochemical Properties of Pork Patties', *Korean journal for food science of animal resources*, 38(5), pp. 889–900. doi: 10.5851/kosfa.2018.e22.

Cuong, T. Van, Thoa, N. T. and THOA, N. T. (2020) 'EFFECT OF HIGH GLUCOSE-INDUCED HYPERGLYCEMIA ON VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV) INFECTION IN ADULT ZEBRAFISH', *Vietnam Journal of Science and Technology*, 58(1), p. 1.

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doi: 10.15625/2525-2518/58/1/13530.

Dong, J. *et al.* (2014) 'Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKα1/SIRT1.', *Journal of Lipid Research*, 55(3), pp. 363–74. doi: 10.1194/jlr.M038786.

Gupta, P. *et al.* (2013) 'Possible modulation of FAS and PTP-1B signaling in ameliorative potential of Bombax ceiba against high fat diet induced obesity', *BMC Complementary and Alternative Medicine*, 13, pp. 1–9. doi: 10.1186/1472-6882-13-281.

Hardie, D. G. (2011) 'AMP-activated protein kinase—an energy sensor that regulates all aspects of cell function', *Genes & Development*, 25(18), pp. 1895–1908. doi: 10.1101/gad.17420111.

Hirata, M. *et al.* (2011) 'Genetic defect in phospholipase Cδ1 protects mice from obesity by regulating thermogenesis and adipogenesis', *Diabetes*, 60(7), pp. 1926–1937. doi: 10.2337/db10-1500.

Howe, K. *et al.* (2013) 'The zebrafish reference genome sequence and its relationship to the human genome', *Nature*, 496(7446), pp. 498–503. doi: 10.1038/nature12111.

Huang, J. *et al.* (2014) 'The anti-obesity effects of green tea in human intervention and basic molecular studies', *European Journal of Clinical Nutrition*. Nature Publishing Group, 68(10), pp. 1075–1087. doi: 10.1038/ejcn.2014.143.

Jeong, G. S., Lee, D. S. and Kim, Y. C. (2009) 'Cudratricusxanthone A from Cudrania tricuspidata suppresses pro-inflammatory mediators through expression of anti-inflammatory heme oxygenase-1 in RAW264.7 macrophages', *International Immunopharmacology*. Elsevier B.V., 9(2), pp. 241–246. doi: 10.1016/j.intimp.2008.11.008.

Kim, H. S. *et al.* (2019) 'Epigallocatechin Exerts Anti-Obesity Effect in Brown Adipose Tissue', *Chemistry and Biodiversity*, 16(10). doi: 10.1002/cbdv.201900347.

Van Der Klaauw, A. A. and Farooqi, I. S. (2015) 'The hunger genes: Pathways to obesity', *Cell*. Elsevier Inc., 161(1), pp. 119–132. doi: 10.1016/j.cell.2015.03.008.

Landgraf, K. *et al.* (2017) 'Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealthy obesity', *BMC Physiology*. BMC Physiology, 17(1), p. 4. doi: 10.1186/s12899-017-0031-x.

Laybutt, D. R. *et al.* (2007) 'Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes', *Diabetologia*, 50(4), pp. 752–763. doi: 10.1007/s00125-006-0590-z.

Lee, J. *et al.* (2016) 'Withaferin A is a leptin sensitizer with strong antidiabetic properties in mice.', *Nature medicine*, 22(9), pp. 1023–1032. doi: 10.1038/nm.4145.

Lin, S. *et al.* (2000) 'Development of high fat diet-induced obesity and leptin resistance in C57B1/6J mice', *International Journal of Obesity*, 24(5), pp. 639–646. doi: 10.1038/sj.ijo.0801209.

Meguro, S., Hasumura, T. and Hase, T. (2015a) 'Body fat accumulation in zebrafish is induced by a diet rich in fat and reduced by supplementation with green tea extract', *PLoS ONE*, 10(3), pp. 1–12. doi: 10.1371/journal.pone.0120142.

Meguro, S., Hasumura, T. and Hase, T. (2015b) 'Body Fat Accumulation in Zebrafish Is Induced by a Diet Rich in Fat and Reduced by Supplementation with Green Tea Extract', *PLOS ONE*. Edited by C. Huang, 10(3), p. e0120142. doi: 10.1371/journal.pone.0120142.

Murase, T. *et al.* (2009) 'Catechin-induced activation of the LKB1/AMP-activated protein kinase pathway', *Biochemical Pharmacology*, 78(1), pp. 78–84. doi: 10.1016/j.bcp.2009.03.021.

Ohyama, K. *et al.* (2011) 'Catechin-rich grape seed extract supplementation attenuates diet-induced obesity in C57BL/6J mice', *Annals of Nutrition and Metabolism*, 58(3), pp. 250–258. doi: 10.1159/000330444.

Oka, T. *et al.* (2010) 'Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity.', *BMC physiology*, 10, p. 21. doi: 10.1186/1472-6793-10-21.

Sáinz, N. *et al.* (2015) 'Leptin resistance and diet-induced obesity: Central and peripheral actions of leptin', *Metabolism: Clinical and Experimental*. Elsevier Inc., 64(1), pp. 35–46. doi: 10.1016/j.metabol.2014.10.015.

Sangeetha, S., Archit, R. and SathiaVelu, A. (2014) 'Phytochemical testing, antioxidant activity, HPTLC

and FTIR analysis of antidiabetic plants Nigella sativa, Eugenia jambolana, Andrographis paniculata and Gymnema sylvestre', *Research Journal of Biotechnology*, 9(9), pp. 65–72.

Sato, S. and Mukai, Y. (2020) 'Modulation of chronic inflammation by quercetin: The beneficial effects on obesity', *Journal of Inflammation Research*, 13, pp. 421–431. doi: 10.2147/JIR.S228361.

Shan, B. *et al.* (2017) 'The metabolic ER stress sensor IRE1α suppresses alternative activation of macrophages and impairs energy expenditure in obesity', *Nature Immunology*, 18(5), pp. 519–529. doi: 10.1038/ni.3709.

Sharma, N. K. *et al.* (2008) 'Endoplasmic reticulum stress markers are associated with obesity in nondiabetic subjects', *Journal of Clinical Endocrinology and Metabolism*, 93(11), pp. 4532–4541. doi: 10.1210/jc.2008-1001.

Shim, J. U. and Lim, K. T. (2009) 'Inhibitory effect of glycoprotein isolated from cudrania tricuspidata bureau on expression of inflammation-related cytokine in bisphenol a-treated HMC-1 cells', *Inflammation*, 32(4), pp. 211–217. doi: 10.1007/s10753-009-9122-6.

Simonds, S. E. *et al.* (2014) 'Leptin mediates the increase in blood pressure associated with obesity', *Cell*. The Authors, 159(6), pp. 1404–1416. doi: 10.1016/j.cell.2014.10.058.

Song, P. *et al.* (2015) 'Apolipoprotein a1 increases mitochondrial biogenesis through AMP-activated protein kinase', *Cellular Signalling*. Elsevier Inc., 27(9), pp. 1873–1881. doi: 10.1016/j.cellsig.2015.05.003.

Subashini, M. S. *et al.* (2015) 'TLC, FTIR and GCMS analysis of leaves of Gymnema sylvestre R. Br from Kolli Hills, Tamil Nadu, India', *Int. J. Curr. Microbiol. App. Sci*, 4(7), pp. 757–764.

Sugumar, N., Karthikeyan, S. and Gowdhami, T. (2015) 'Chemical Composition and Antimicrobial Activity of Essential Oil from *Eupatorium triplinerve* Vahl. Aerial Parts', *International Letters of Natural Sciences*, 31(3), pp. 14–21. doi: 10.56431/p-6l0v4s.

Thoa, N. and Cuong, T. (2018) 'Phytochemical Components, Antioxidant and Cytotoxic Activities of Mulberry Mistletoe (Loranthus parasiticus Merr) Leaves Extracts', *Asian Journal of Biotechnology and Bioresource Technology*, 2(4), pp. 1–11. doi: 10.9734/AJB2T/2017/39166.

Tian, Y. H. *et al.* (2005) 'Hepatoprotective constituents of Cudrania tricuspidata', *Archives of Pharmacal Research*, 28(1), pp. 44–48. doi: 10.1007/BF02975134.

Tran, V. C. *et al.* (2019) 'Alginate oligosaccharide (AOS) improves immuno-metabolic systems by inhibiting STOML2 overexpression in high-fat-diet-induced obese zebrafish', *Food & Function*. Royal Society of Chemistry, 10(8), pp. 4636–4648. doi: 10.1039/C9FO00982E.

Wu, L. *et al.* (2018) 'AMP-Activated Protein Kinase (AMPK) regulates energy metabolism through modulating thermogenesis in adipose tissue', *Frontiers in Physiology*, 9(FEB), pp. 1–23. doi: 10.3389/fphys.2018.00122.

Yan, J. Q., Zhao, Y. and Zhao, B. L. (2013) 'Green tea catechins prevent obesity through modulation of peroxisome proliferator-activated receptors', *Science China Life Sciences*, 56(9), pp. 804–810. doi: 10.1007/s11427-013-4512-2.