國立嘉義大學生命科學院

學生學術研究成果優良海報評選獲獎名單

時間:106 年 5 月 24 日

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Investigate the skin whitening and anti-aging effect of Taiwan rice cultivar: TNGSW26

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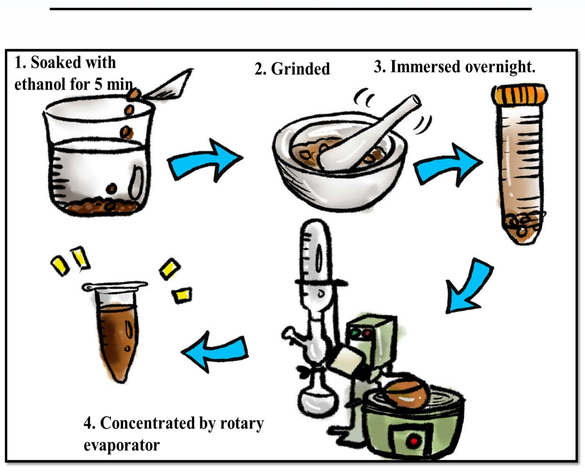
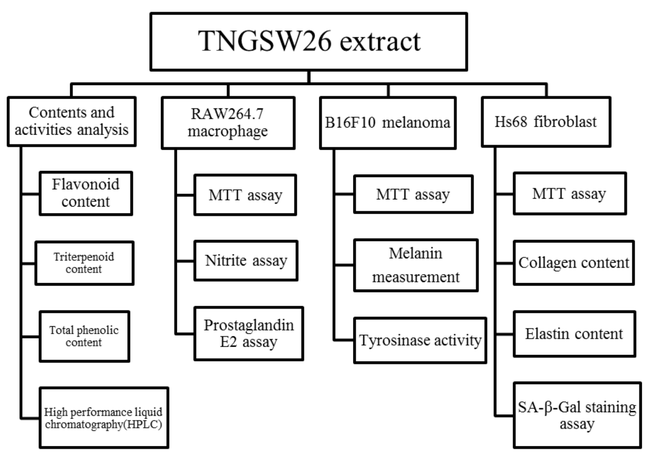
Introduction

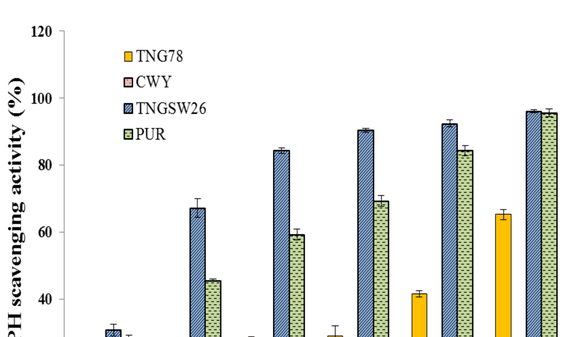
TNGSW26 is a new improved varieties which come from Chiayi Agricultural Experiment Branch.
Rice is the most widely consumed staple food for a large part of the world‘s human population, especially in Asia. Rice exists in different colors such as white, purple, black, red and brown. Although white rice is the most widely consumed rice, pigmented rice is considered as enriched rice for taste and health benefits. At present, several new types of rice are now available in food markets. According to the previous researches: Pigmented rice have a higher content of phenolic and flavonoid compounds as compared to white rice (Yawadio et al., 2007). Phetpornpaisan et al. isolated and analyzed purple rice and then which presented high antioxidant activity (Phetpornpaisan et al.,2014) . In addition, there are several rice-containing cosmetic products in the market.
Therefore, we are interest to isolate and analyze bio-active compounds of local Taiwan rice strain: TNGSW26. After the measurement of anti-oxidant activity in ethanol extract of TNGSW26, the anti-inflammatory, skin whitening and anti-aging effects for ethanol extract of TNGSW26 were determined.

Materials and Methods





Results



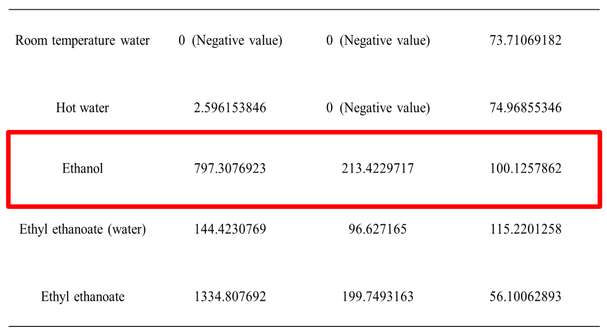


Figure 1. DPPH scavenging activity of TNGSW26.

	Flavonoid content (μg/g)	Triterpenoid content (μg/g)	Total phenolic content (μg/g)
Room temperature water	0 (Negative value)	0 (Negative value)	73.71069182
Hot water	2.596153846	0 (Negative value)	74.96855346
Ethanol	797.3076923	213.4229717	100.1257862
Ethyl ethanoate (water)	144.4230769	96.627165	115.2201258
Ethyl ethanoate	1334.807692	199.7493163	56.10062893

Table 1. Total phenolic, flavonoid, and Triterpenoid content of different TNGSW26 extracts.

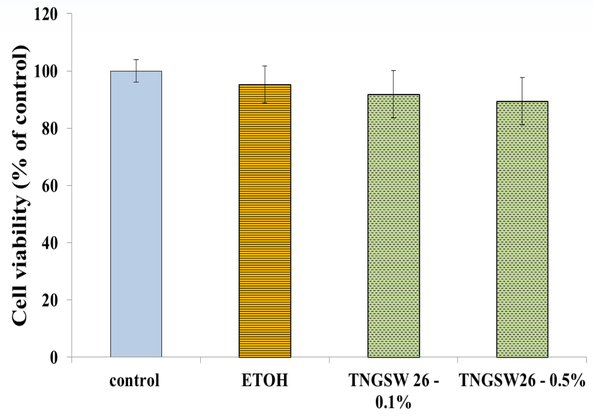


Figure 2. The effect of TNGSW26 on cell viability of RAW264.7 mouse macrophages.

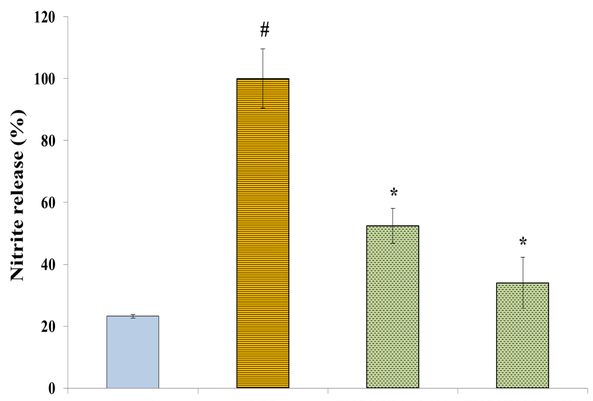


Figure 3. The inhibition of NO-production by TNGSW26 extract in LPS-induced RAW264.7 mouse macrophages. (# p<0.05 with respect to control * p<0.05 with respect to cells treated with LPS)

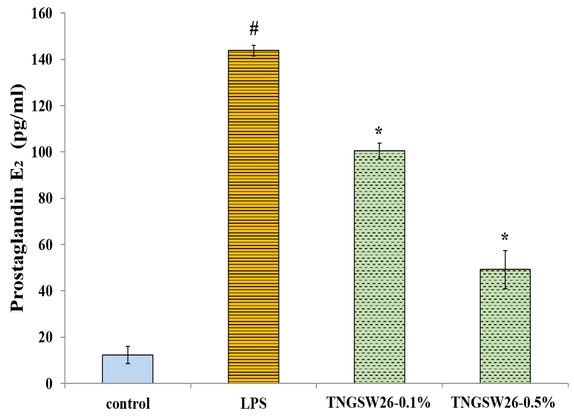


Figure 4. The anti-inflammatory activity of TNGSW26 extract in LPS-induced RAW264.7 mouse macrophages. (# p<0.05 with respect to control * p<0.05 with respect to cells treated with LPS)

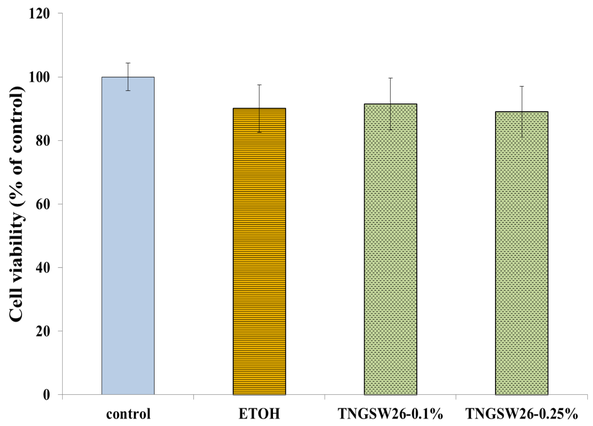


Figure 5. The effect of TNGSW26 extract on cell viability of B16F10 mouse melanoma cells

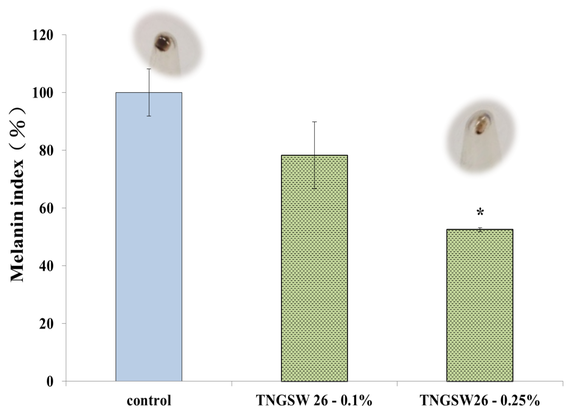


Figure 6. The anti-melanogenic effects of TNGSW26 extract on B16F10 mouse melanoma cells. (* p<0.05 with respect to cells treated with control)

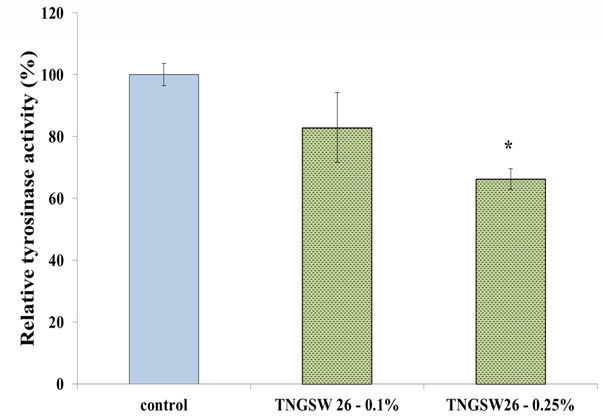


Figure 7. The effect of TNGSW26 extract on tyrosinase activities of B16F10 mouse melanoma cells (* p<0.05 with respect to cells treated with control)

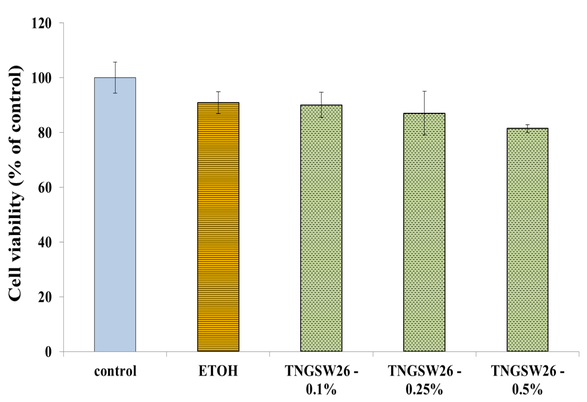


Figure 8. The effect of TNGSW26 extract on cell viability of Hs68 fibroblasts

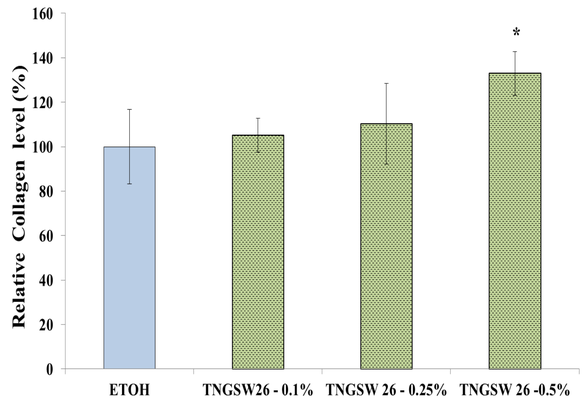


Figure 9. The stimulating effect of TNGSW26 extract on collagen synthesis. (* p<0.05 with respect to cells treated with control)

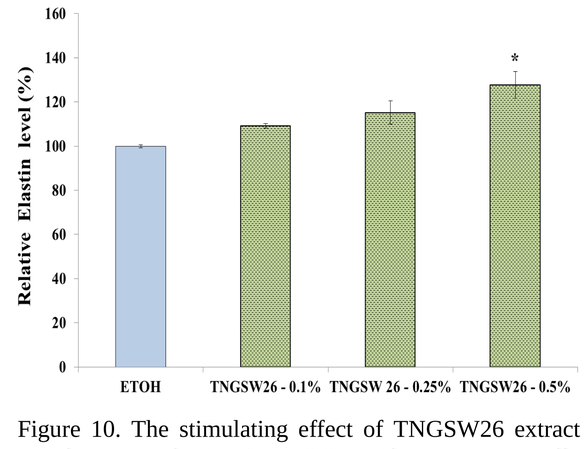


Figure 10. The stimulating effect of TNGSW26 extract on elastin synthesis. (* p<0.05 with respect to cells treated with control)

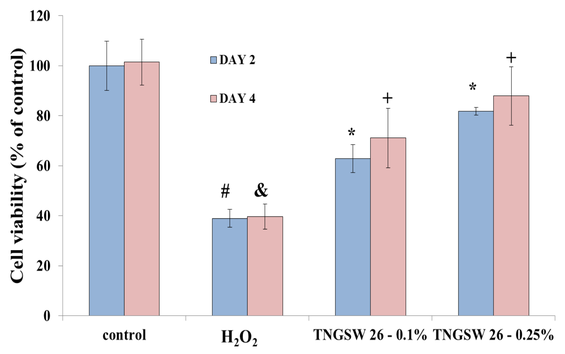
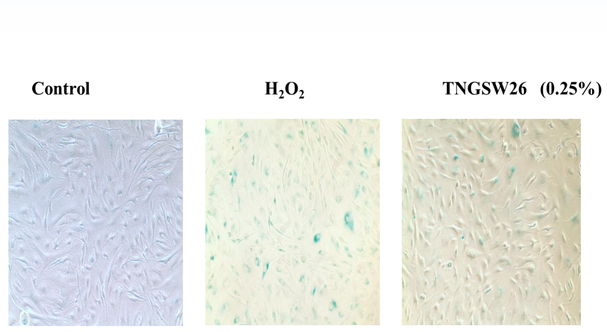


Figure 11. The effect of TNGSW26 extract on cell proliferation of H_2O_2-treated Hs68 human dermal fibroblasts. (DAY2: # p<0.05 with respect to control * p<0.05 with respect to cells treated with H_2O_2. & p<0.05 with respect to control. DAY4: + p<0.05 with respect to cells treated with H_2O_2.)



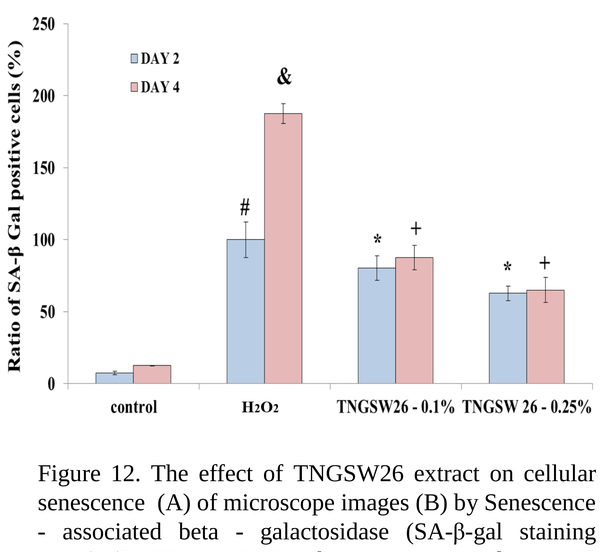


Figure 12. The effect of TNGSW26 extract on cellular senescence (A) of microscope images (B) by Senescence - associated beta - galactosidase (SA-β-gal staining assay). (DAY2: # p<0.05 with respect to control * p<0.05 with respect to cells treated with H_2O_2. & p<0.05 with respect to control. DAY4: + p<0.05 with respect to cells treated with H_2O_2.)

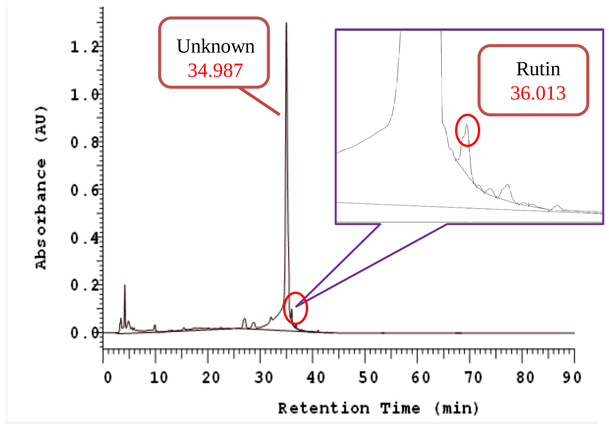


Figure 13. HPLC profile of TNGSW26 extract.

Conclusion

➢ The ethanol extract of TNGSW26:

- ✓ Good anti-oxidant and anti-inflammatory activity.
- ✓ Inhibit melanin synthesis and tyrosinase activity in B16F10 melanoma cells.
- ✓ Stimulate collagen and elastin synthesis in Hs68 human dermal fibroblasts.
- ✓ Inhibit H_2O_2-induced cell senescence in Hs68 human dermal fibroblasts.

Good cosmetic product

REFERENCES

Phetpornpaisan, P., Tippayawat, P., Jay, M., & Sutthanut, K. A local Thai cultivar glutinous black rice bran: A source of functional compounds in immunomodulation, cell viability and collagen synthesis, and matrix metalloproteinase-2 and-9 inhibition. Journal of Functional Foods, 7, 650-661, 2014.

探討南瓜複方萃取液對 STZ 誘發糖尿病大鼠餵食高脂飲食的血糖及血脂之影響

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一、摘要

糖尿病（DM）是世界上最重要的慢性疾病之一。血糖控制不良會增加糖尿病相關併發症（如心血管疾病、腎臟疾病、神經病、失明和下肢截肢）的風險。南瓜（中國南瓜）屬於葫蘆科，是世界知名的食材，通常用作台灣的主食，果肉、果皮和籽都可食用，它含有豐富的碳水化合物、纖維素、維生素以及一些蛋白質和脂質，可預防高血壓、糖尿病及肝臟病變，提高人體免疫能力。

除了對照組外，經由腹腔注射菸鹼醯胺與 STZ 誘導糖尿病並餵食高脂肪飲食（HFD）的大鼠發展為第 2 型糖尿病。四周後，結果顯示超音波南瓜複方萃取液組的飯前血糖濃度明顯降低。用南瓜複方萃取物治療的糖尿病大鼠顯示血清中三酸甘油酯、總膽固醇含量顯著降低。總之，超音波複方萃取液明顯改善了第 2 型糖尿病大鼠的飯前血糖和降低血脂。

二、材料與方法

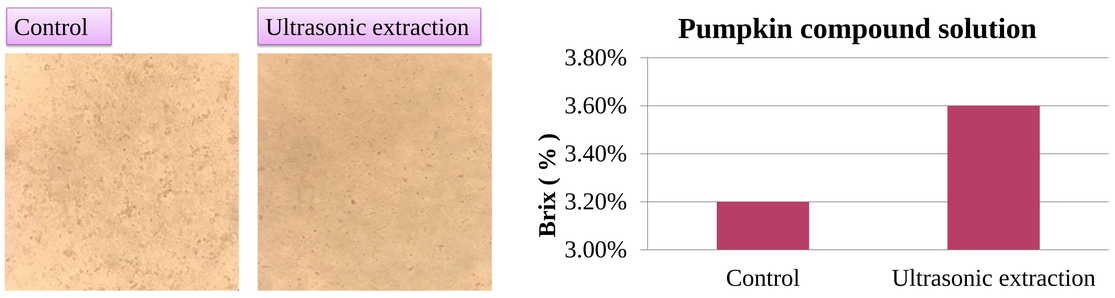
2.1 實驗動物

6 週齡 Sprague-Dawley（SD）雄性大白鼠

2.2實驗材料

南瓜（中國南瓜）

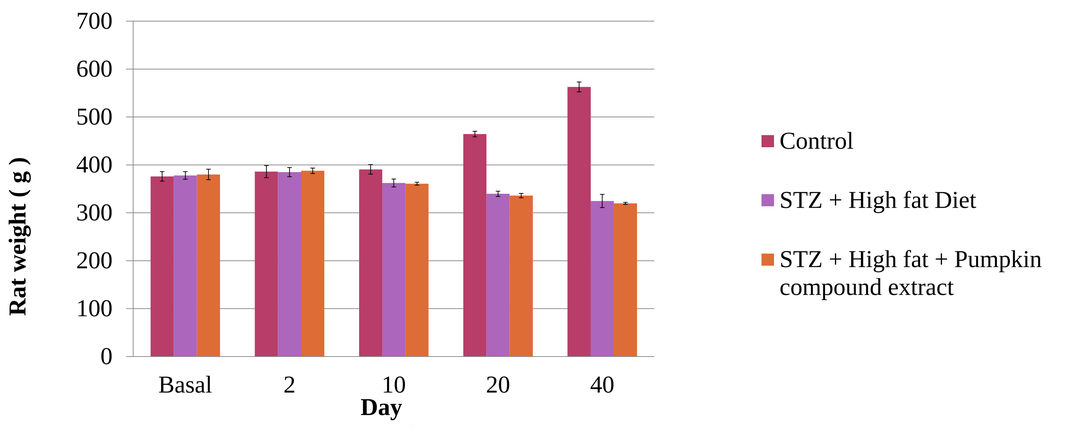
三、結果與討論



圖一、超音波南瓜複方萃取液與未萃之分子大小及糖度變化

左圖可以明顯看出未萃取南瓜複方液的物質顆粒較大，經過萃取後的南瓜複方液的物質顆粒較小；右圖未萃取南瓜複方液的 Brix 為 3.2 %，但經過超音萃取震盪後，Brix 升高為 3.6 %，經過萃取後造成顆粒變小、糖度變高，明顯看出南瓜複方液經過超音波萃取後的改變。

進行大鼠動物實驗將 Sprague-Dawley（SD）分為對照組、STZ 誘發糖尿病大鼠+餵食高脂飼料組以及 STZ 誘發糖尿病大鼠+餵食高脂飼料+南瓜複方萃取液組，使用 STZ 成功誘發糖尿病後，每天餵食高脂飼料，並且另一糖尿病鼠組加上每天餵食南瓜複方萃取液。

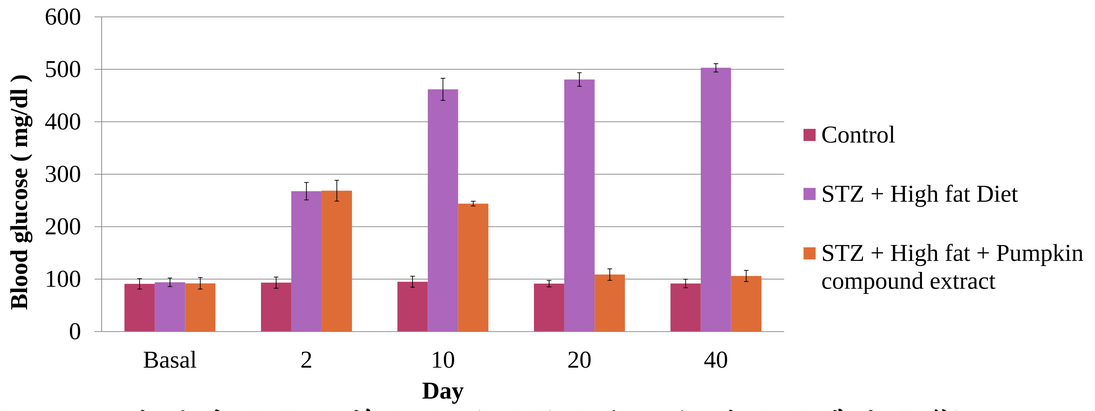


圖二、攝取超音波南瓜複方萃取液兩個月對糖尿病大鼠之體重影響

對照組因為正常飼養，所以體重會隨著飼養天數逐漸增加，而因 STZ 誘導下的第二型糖尿病鼠並餵食高脂飼料，體重會逐漸下降，並在第 40 天後體重在 325 ± 14 g，與 STZ 誘發糖尿病大鼠 + 高脂飼料 ＋ 餵食南瓜複方萃取液組也呈現這樣的結果。

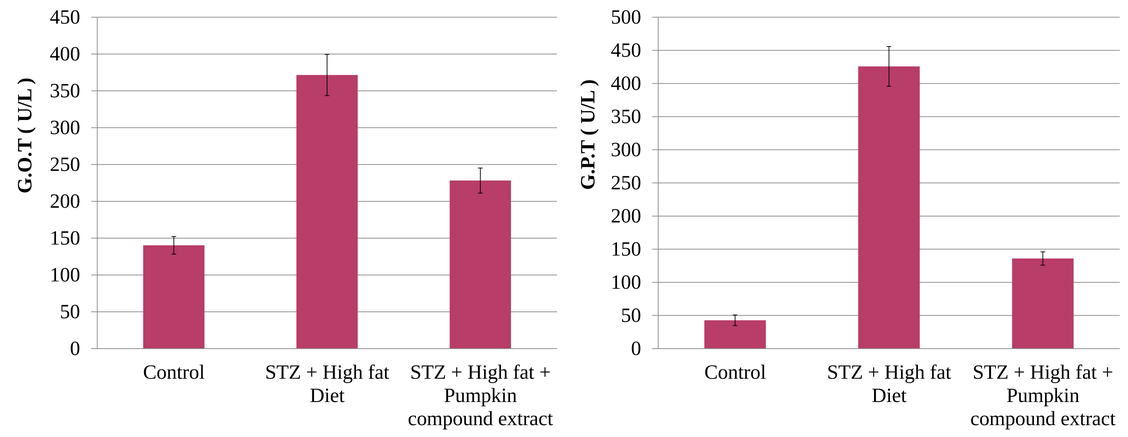
圖三、攝取超音波南瓜複方萃取液對糖尿病大鼠之尿液影響

可觀察出對照組墊料乾燥，且排便量正常；而 STZ 誘發糖尿病大鼠+餵食高脂飼料組之墊料為潮濕狀態，並且大便量多、濕軟；但經過 STZ 誘發糖尿病大鼠+高脂飼料+餵食南瓜複方萃取液可發現墊料狀態呈現乾燥，且大便量為正常，大便為硬顆粒型。

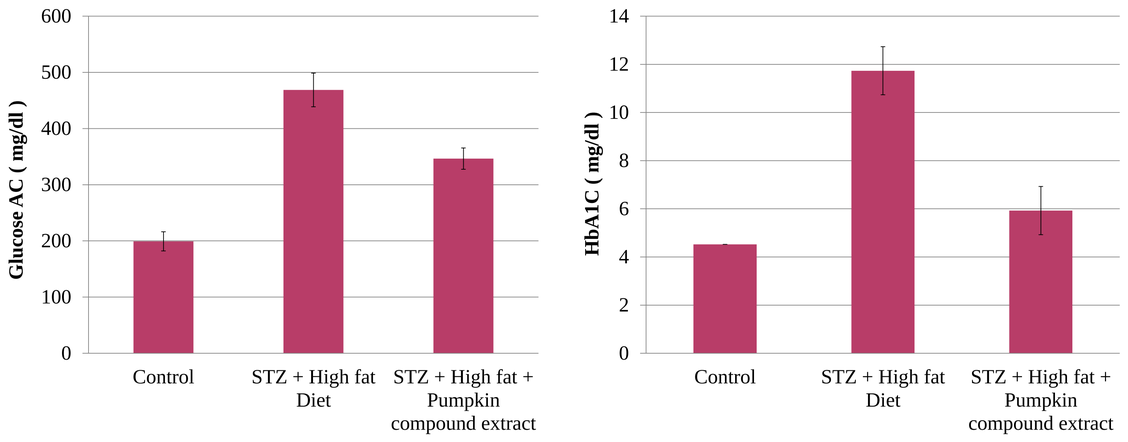


圖四、超音波南瓜複方萃取液對糖尿病鼠之飯前血糖濃度影響

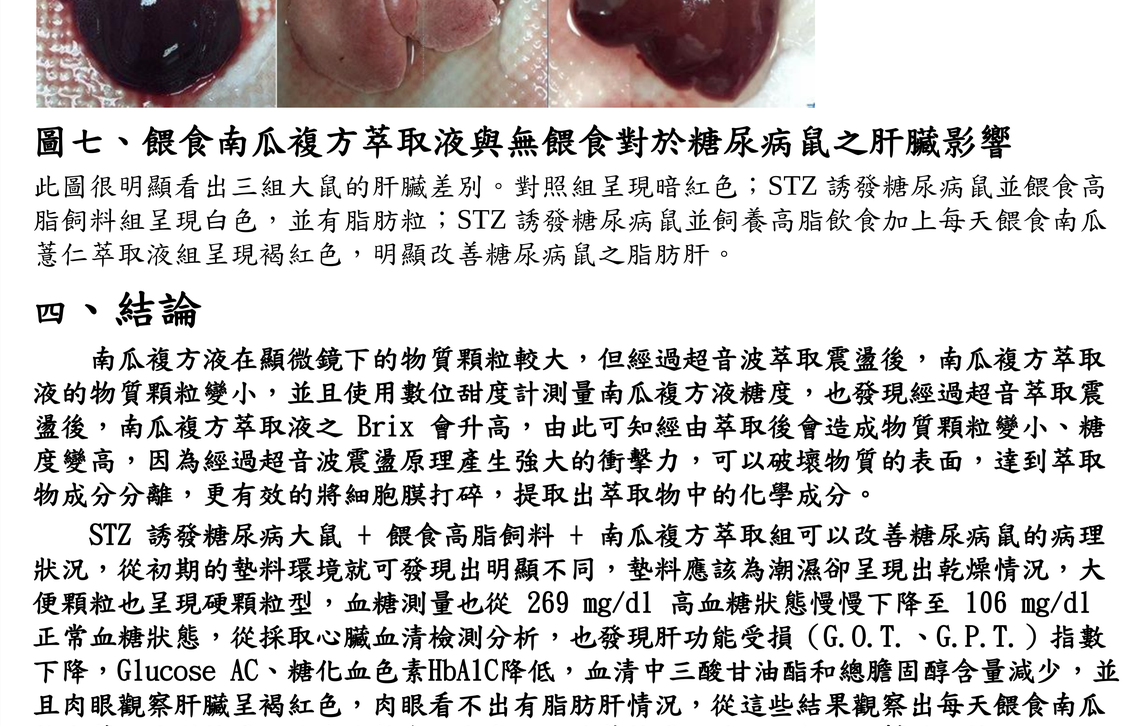
可看出 STZ 誘發糖尿病大鼠 + 餵食高脂飼料組為高血糖狀態；但經由 STZ 誘發糖尿病大鼠及餵食高脂飼料並加上每天餵食南瓜複方萃取液可使糖尿病鼠之高血糖降低，呈現正常狀態。



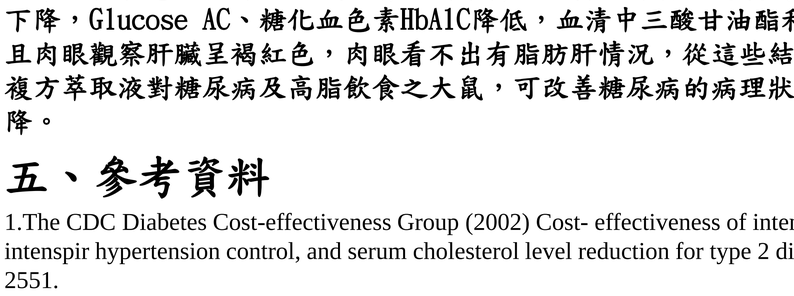
圖五、餵食南瓜複方萃取液與無餵食對於糖尿病大鼠之肝功能影響



圖六、餵食南瓜複方萃取液對糖尿病大鼠之血糖及糖化血色素的影響



圖六、餵食南瓜複方萃取液可改變糖尿病鼠之三酸甘油酯及總膽固醇



圖七、餵食南瓜複方萃取液與無餵食對於糖尿病鼠之肝臟影響

此圖很明顯看出三組大鼠的肝臟差別。對照組呈現暗紅色；STZ 誘發糖尿病鼠並餵食高脂飼料組呈現白色，並有脂肪粒；STZ 誘發糖尿病鼠並飼養高脂飲食加上每天餵食南瓜薏仁萃取液組呈現褐紅色，明顯改善糖尿病鼠之脂肪肝。

四、結論

南瓜複方液在顯微鏡下的物質顆粒較大，但經過超音波萃取震盪後，南瓜複方萃取液的物質顆粒變小，並且使用數位甜度計測量南瓜複方液糖度，也發現經過超音萃取震盪後，南瓜複方萃取液之 Brix 會升高，由此可知經由萃取後會造成物質顆粒變小、糖度變高，因為經過超音波震盪原理產生強大的衝擊力，可以破壞物質的表面，達到萃取物成分分離，更有效的將細胞膜打碎，提取出萃取物中的化學成分。

STZ 誘發糖尿病大鼠 + 餵食高脂飼料 + 南瓜複方萃取組可以改善糖尿病鼠的病理狀況，從初期的墊料環境就可發現出明顯不同，墊料應該為潮濕卻呈現出乾燥情況，大便顆粒也呈現硬顆粒型，血糖測量也從 269 mg/dl 高血糖狀態慢慢下降至 106 mg/dl 正常血糖狀態，從採取心臟血清檢測分析，也發現肝功能受損（G.O.T.、G.P.T.）指數下降，Glucose AC、糖化血色素HbA1C降低，血清中三酸甘油酯和總膽固醇含量減少，並且肉眼觀察肝臟呈褐紅色，肉眼看不出有脂肪肝情況，從這些結果觀察出每天餵食南瓜複方萃取液對糖尿病及高脂飲食之大鼠，可改善糖尿病的病理狀況，幫助血糖、血脂下降。

五、參考資料

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Synergistic effects of chrysin and pinocembrin combination with paclitaxel on inhibition of melanoma cells

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Introduction

Malignant melanoma is a serious type of skin cancers, which develops from the pigment-containing cells like melanocytes. The melanoma has high metastasis and invasion activities, and then causes patients with high death rate. The treatment of melanoma in clinical includes excision tumor, chemotherapy, radiation, and immunotherapy. However, the treatment effects were poor and the regulatory mechanisms are still unclear. Natural compounds, chrysin and pinocembrin, are the major integrates of pine tree (*Pinus morrisonicola* Hayata), which were reported with the activities of anti-inflammation, anti-oxidation, and enhancing the inhibition of cancer cells. The aim of this study assessed the effects of chrysin and pinocembrin combination with paclitaxel on inhibition of murine B16-F10 melanoma cells and clarified the possible regulatory molecules. Cell viability assay by trypan blue dye method showed that chrysin and piocembrin alone were down-regulated, as well as the combination with paclitaxel enhanced such effect. Chrysin and pinocembrin combined treatment with paclitaxel also increase the percent of Sub-G1 phase and melanin production in melanoma B16 cells. Wound healing assay demonstrated the chrysin and pinocembrin decrease the level of cell migration, and combination treatment with paclitaxel has synergistic effect. Moreover, the regulation molecules including metastasis (E-, N-cadherin, and MMP-9) and apoptosis (caspase-9 and β-catenin) will be further assayed by Western blot analysis. The results demonstrated that there are synergistic effect of chrysin and pinocembrin with the combination of paclitaxel on down-regulating cell viability, inducing apoptosis, and inhibiting cell migration in melanoma B16 cells. Conclusions, the present study provide several information of natural product chrysin and pinocembrin for enhancing the anti-cancer ability in melanoma cells.

Method

Chrysin、Pinocembrin, and combined Paclitaxel

↓

Melanoma B16-F10 cells

↓

Cell viability
-Trypan blue exclusion test

Cell cycle assay

Wound healing assay

Cell Morphology

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Synergistic effect on anti-melanoma

Results & Discussion

1. Chrysin, pinocembrin, and combined paclitaxel decreased the viability of B16-F10 cells:

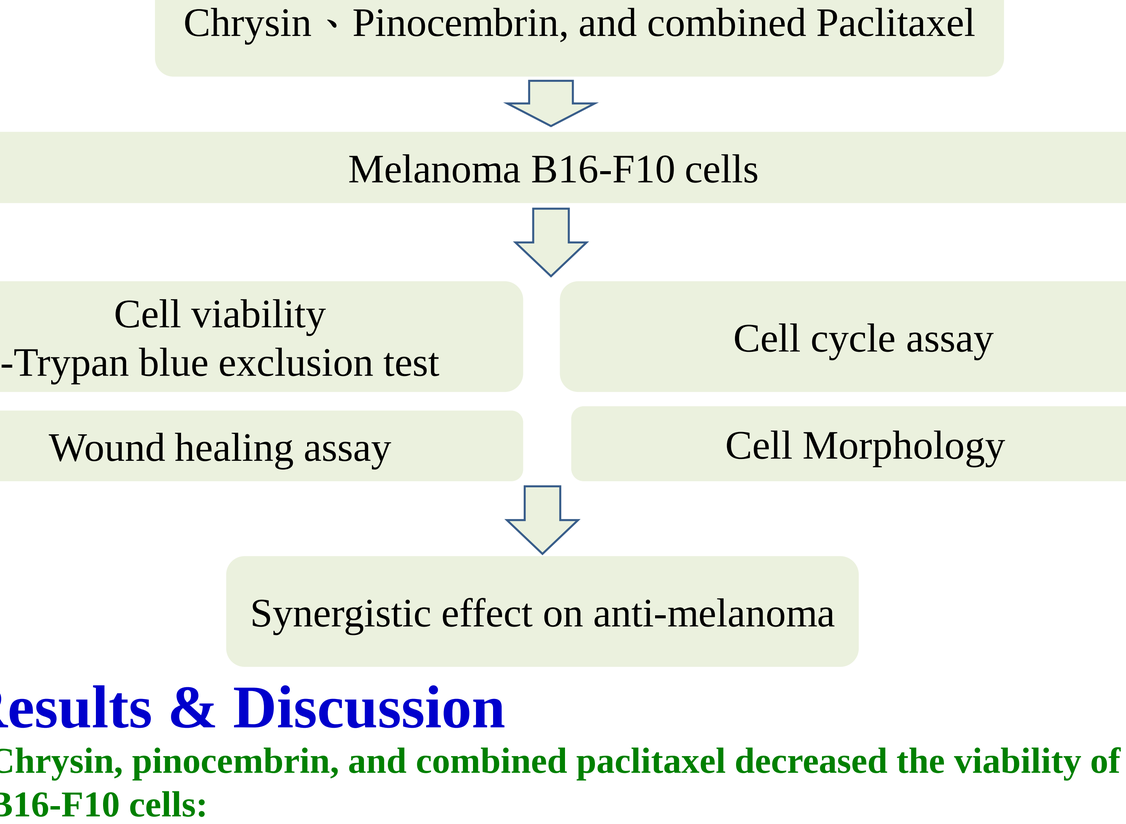


Figure 1： Viability of chrysin, pinocembrin, and combined paclitaxel and treatment for 24 and 48 h in B16-F10 cells. * P< 0.05, ** P<0.01, compared with control, # P< 0.05, ## P< 0.01, compared with paclitaxel.

2. Chrysin, pinocembrin, and combined paclitaxel caused morphological changes of cells:

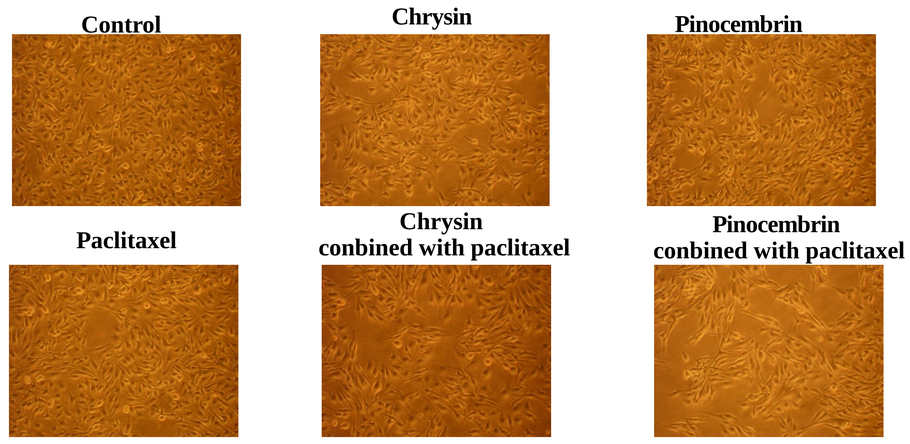


Figure 3： Morphological changes of B16-F10 cells after chrysin, pinocembrin, paclitaxel alone, and chrysin, pinocembrin combined paclitaxel and treatment for 48 h in B16-F10 cells.

3. Chrysin, pinocembrin, and combined paclitaxel increased the level of Sub-G1 phase:

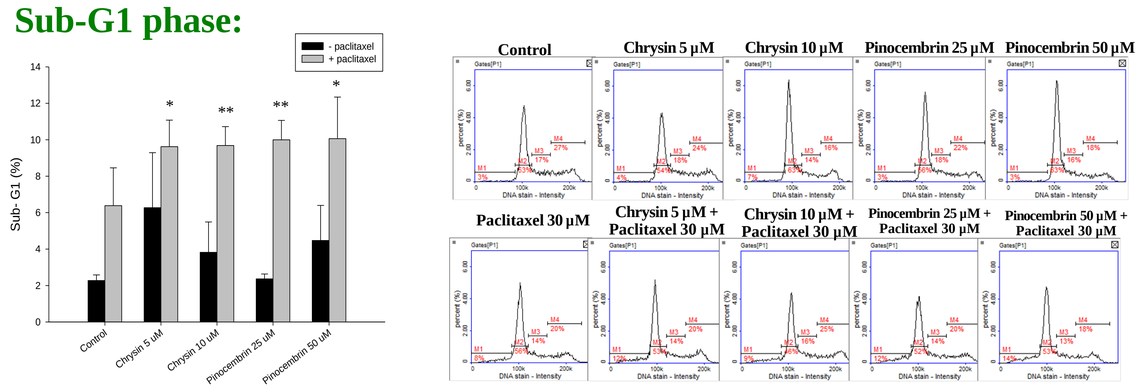


Figure 4： Cell cycle analysis of chrysin, pinocembrin, paclitaxel, and chrysin, pinocembrin combined paclitaxel and treatment for 24 h. * P< 0.05, ** P<0.01, compared with control, # P< 0.05, compared with paclitaxel.

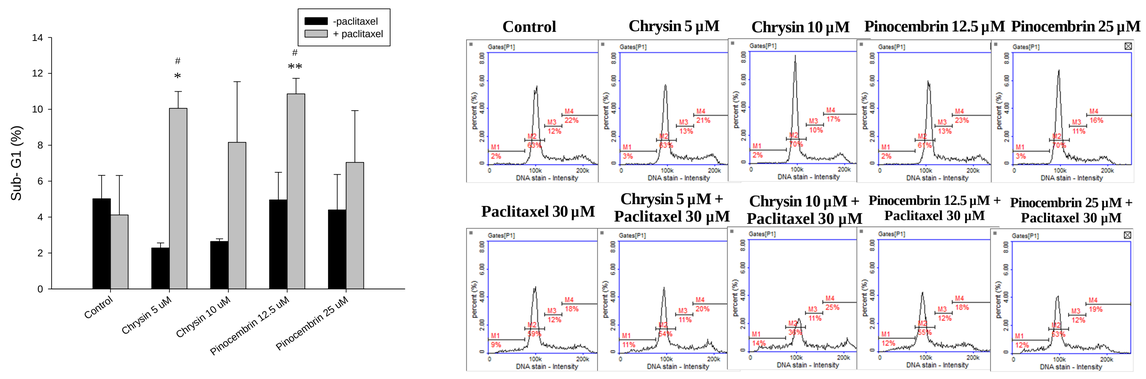
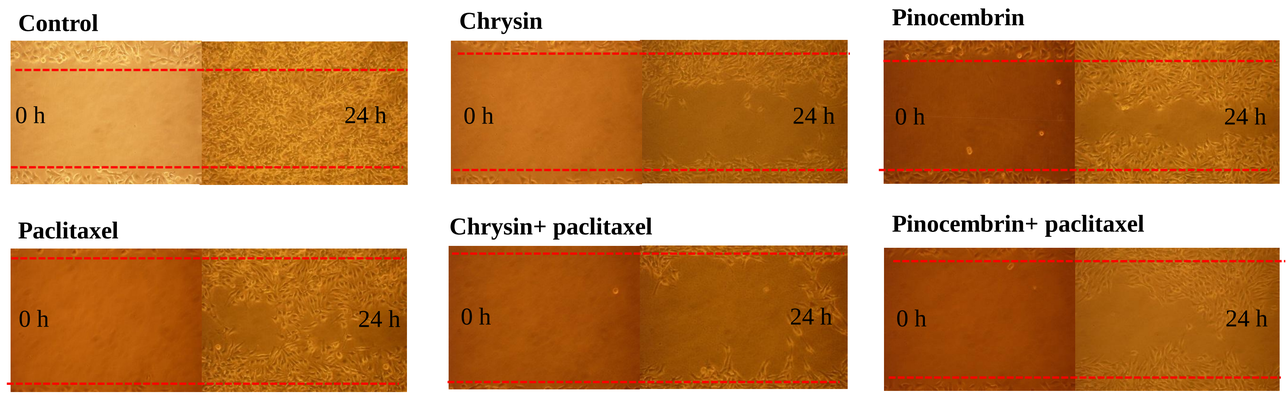


Figure 5： Cell cycle analysis of chrysin, pinocembrin, paclitaxel, and chrysin, pinocembrin combined paclitaxel and treatment for 48 h. * P< 0.05, ** P<0.01, compared with control, # P< 0.05, compared with paclitaxel.

4. Chrysin, pinocembrin, and combined paclitaxel decreased cell migration:

(A)



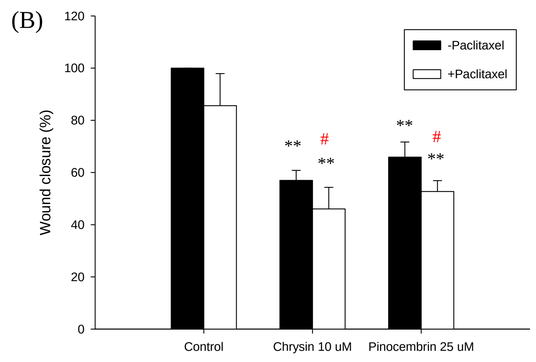


Figure 6： Wound healing assay of chrysin, pinocembrin, paclitaxel, and chrysin, pinocembrin combined paclitaxel and treatment for 24 h. (A) Morphological (B) Wound closure percentage. * P< 0.05, ** P<0.01, compared with control, # P< 0.05, compared with paclitaxel.

Conclusions

1. Chrysin and pinocembrin have synergistic effects to enhance anti-melanoma effect of paclitaxel on decrease viability, change cell morphology, increase Sub-G1 phase, and decrease cell migration in melanoma B16 cells for 24 and 48 h-treatment.
2. The present study provide several information of natural product chrysin and pinocembrin on anti-cancer activity in melanoma cells.