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Study of water and ethanol extracts from *Ganoderma lucidum* on antioxidant and whitening effects by a zebrafish embryo model system

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Ganoderma lucidum is a traditional Chinese medicinal fungi, the main bioactive compounds are polysaccharides and triterpenoids which were microwave extracted by hot water and ethanol, respectively. The objective of this study was to utilize the phenotype-based zebrafish model to verify the water and ethanol extracts from *Ganoderma lucidum* on antioxidant and whitening effects. Both 50~200 ppm water extracts and 100~200 ppm ethanol extracts from G. *lucidum* significantly protected zebrafish embryo from damage of hydrogen peroxide induced oxidative stress; however, water extracts had better antioxidant activities than ethanol extracts. Water extract (200ppm) from G. *lucidum* exhibited tyrosinase inhibition of 34%, which was significantly higher than 50~200 ppm ethanol extracts from G. *lucidum*. In addition, 200 ppm water extract from significantly decreased melanogenesis of zebrafish embryos treated with melanogenic regulators at 78 hpf than ethanol extracts from *G. lucidum*. Therefore, the antioxidant and whitening effects of extracts from G. *lucidum* can be verified by the zebrafish embryo model system.
Keywords*: Ganoderma lucidum*, zebrafish, antioxidant, whitening

Introduction

Ganoderma lucidum has several pharmacological effects such as immunomodulatory, anti-inflammatory, anti-oxidation, anti-viral, anti-bacterial, anti-tumor, hypoglycemic, liver protection and skin whitening etc. According to both the extracts of *G. lucidum* (Chien *et al*., 2008) and adlay (Huang *et al*., 2014) inhibited tyrosinase activity and they had whitening effects; therefore, the *G. lucidum* solid-state 2-weeks fermented red adlay product was produced. The 1 kg *G. lucidum* fermented product was pasteurized and dried by radio frequency (RF) heating with 11 cm gap for 45 s and 8 min, respectively, to instead of long time autoclaving and hot air drying. For the zebrafish embryo model system of antioxidant activities and whitening effects of G. *lucidum* extracts were verificated (Choi *et al*., 2007).

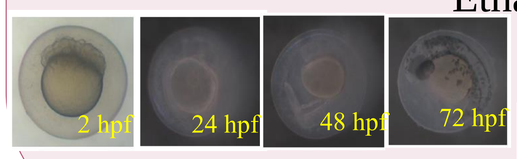
Experimental design

Ganoderma lucidum (BCRC36123) pre-activation
↓ 2-weeks fermentation at 30℃
1 kg *Ganoderma lucidum* solid-state fermented red adlay product

↓ RF pasteurization at gap of 11 cm (45 s)
RF drying at gap of 11 cm (8 min)

↓ Grinding (60 mesh)
↓ 1000 W microwave extraction 10 min
↓ 1:10 Ethanol or Water

Water extracts (WE)
Ethanol extract (EE)
↓ **Zebrafish embryo**



Antioxidant **Whitening effects**

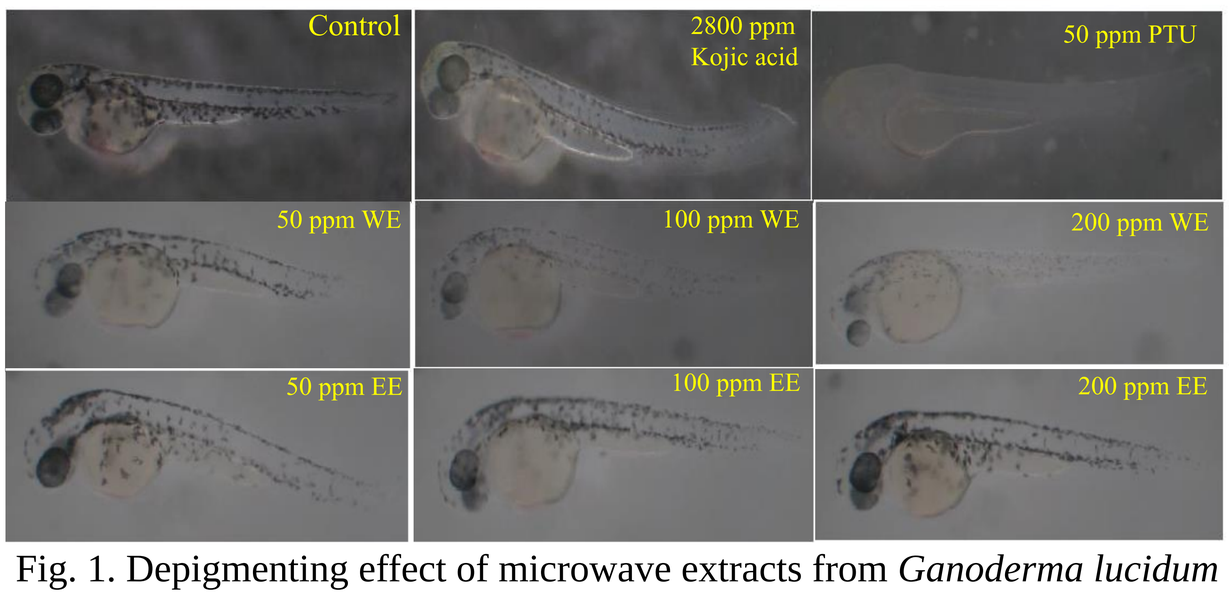


Fig. 1. Depigmenting effect of microwave extracts from *Ganoderma lucidum* fermented red adlay product on melanogenesis of zebrafish embryos treated with melanogenic regulators at 78 hpf (72 hpf treatment).

Table 1. The antioxidant activities of extracts from *G. lucidum* fermented product

20 mg/mL	Scavenging DPPH free radicals (%)	Reducing power
WE	85.36 ± 0.08^{b}	1.56 ± 0.02^{d}
EE	77.36 ± 2.26^{c}	0.52 ± 0.02^{c}
Ascorbic acid	91.97 ± 0.05^{a}	1.91 ± 0.04^{b}
BHA	93.00 ± 0.05^{a}	2.05 ± 0.09^{a}

Results

The water extract (WE) and ethanol extract (EE) from *G. lucidum* fermented products had antioxidant activities (Table 1, Fig. 2). The whitening effects of zebrafish embryos by immersing different concentration of WE and EE were showed in Fig. 1. The inhibition of tyrosinase and melanin synthesis of zebrafish embryo by WE and EE were showed in Fig. 3 and Table 2, respectively.

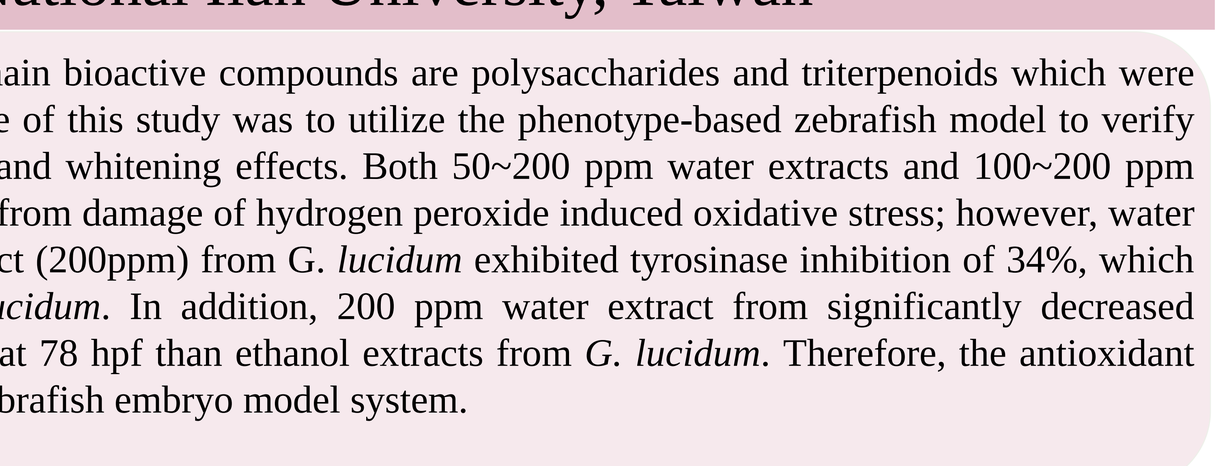


Fig. 2. The protective effect water and ethanol extracts from *G. lucidum* fermented product on hydrogen peroxide induced oxidative stress in zebrafish embryos.

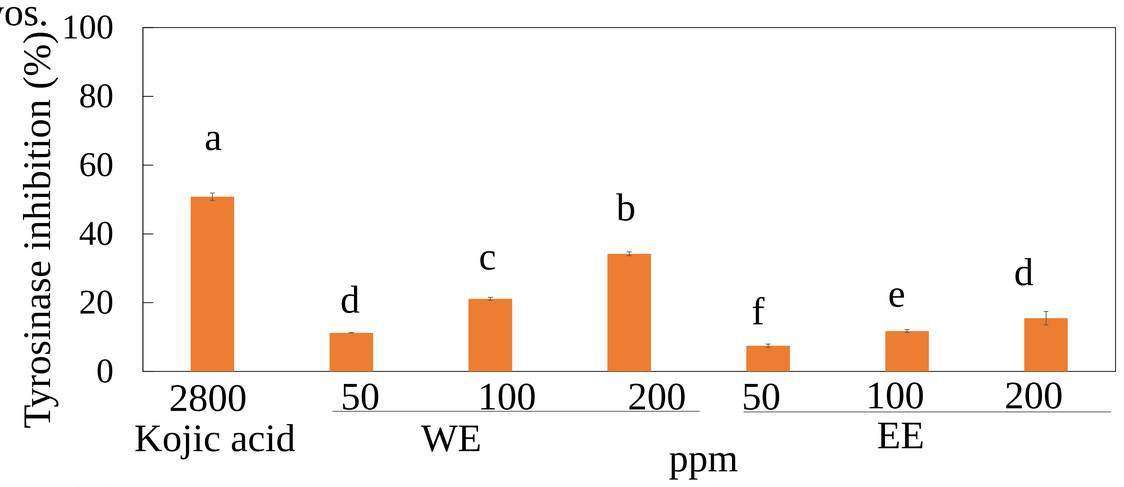


Fig. 3. Inhibitory effect of water extract (WE) and ethanol extract (EE) from *G. lucidum* fermented product on the tyrosinase activity.

Table 2. Inhibitory effect of water and ethanol extracts from *G. lucidum* and melanogenic regulator on the melanin

	Relative melanin content (% of control)
Control	100.0 ± 0.02
Kojic acid 500 ppm	80.5 ± 0.02
WE 200 ppm	70.7 ± 0.01
EE 200 ppm	93.8 ± 0.03

Conclusions

The water extract (WE) had better antioxidant activities than ethanol extract (EE) from *G. lucidum* solid-state fermented red adlay product. In addition The WE had better whitening effects than EE by inhibiting tyrosinase activity and melanin synthesis of zebrafish embryos, so it can be used in skin care cosmetics.

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