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Antioxidant and antigenotoxic effects of pupae of the muga silkworm *Antheraea assamensis*

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ABSTRACT

This study investigated the antioxidant, antityrosinase and antigenotoxic ability of pupae of the muga silkworm *Antheraea assamensis*. DPPH radical scavenging activity of methanolic pupae extract (MPE) with IC_{50} value of 25.83 μg/mL was highly correlated with MPE of in vitro lipid peroxidation activity ($r=0.985$, $p>0.05$) and antityrosinase activity ($r=0.894$, $p<0.05$). The MPE had significant higher phenolic (11.2 mg catechin/g) and flavanoid content (5.12 mg quercetin/g) ($p<0.05$). The major fatty acid compounds found in the pupae of the muga silkworm were palmitic acid (23.36%), oleic acid (15.31%), stearic acid (5.83%), linoleic acid (2.76%) and palmitoleic acid (0.73%). At concentration of 1 mg/mL, the pupae extract exhibit the protective effect on H_2O_2 induced DNA damage by Comet assay. The mineral contents of pupae were Al (9.67 mg/g), Ca (0.809 mg/g), K (0.59 mg/g), Mg (0.55 mg/g), and Zn (0.54 mg/g). Therefore, pupae could be used as natural antioxidants on food products.

1. Introduction

Insects are widely used for their potential source of protein, lipids, carbohydrates and certain vitamins in many parts of the world (Ozdal et al., 1997). Among the insects, silkworms are well known as an efficient large-scale producer of silk threads. Among the various species of silkworms, the mulberry and non mulberry silkworm are of common use in sericulture (Zhou & Han, 2006). In the process of cooking and reeling of cocoons, certain byproducts are obtained. The chemical composition and the possible uses of this industrial byproduct were studied by many researchers in the past (Datta & Chaterji, 1997; Kipriotis, Penkov, Grekov, & Ivanov, 2000). The pupae of mulberry and non-mulberry silkworm were traditionally accepted and used as fertilizer, animal feed and edible insects in some countries, such a Japan, Korea, India, and Thailand (Pereira, Ferrarese-Filho, Matsushita, & De-Souza, 2003). In China, human consumption of silkworm pupae has been practiced since the very earliest times (Zhou & Han, 2006). The pre-pupae and pupae of the silkworm are considered as a delicacy in northeast India; however the harvesting and sale of the silkworm pre-pupae and pupae is still informal and it is sold only in local markets (Longvah, Mangthya, & Ramulu, 2011). The killed pupa represents approximately about 43–50% of the total dry cocoon weight, at the stage they are driven to reeling factories (Tzenov & Zaharieva, 1997). Wattanathron et al. (2012) reported that the silkworm pupae *of Bombyx mori* protect against Alzheimer's disease. Consumption of silkworm pupae could supplement vitamin B_2 intake, which can be important to prevent the serious effects of vitamin B_2 deficiency (Kwon et al., 2012). Recently, it has been reported that fermented silkworm powder has a protective effect in alcohol-induced hepatotoxicity in a rat model (Cha, Kim, Moon, & Cho, 2012).

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It has also been reported that silkworm pupae can significantly raise hemoglobin and serum total protein in rats, producing protective effects on the liver in carbon tetrachloride-induced rat hepatic injury (Shi, Xie, Zhang, & Shen, 1990; Yang, Zhu, & Lu, 2002). Information on consumption of silkworm pupae in some countries is available, with limited data on health beneficial effects. In China, the use of silkworm pupae as food has been practiced since ancient times and silkworm has been approved as a new food source by the Ministry of Health of the Republic of China (Zhu, 2004). The pupae of this semi domesticated silkworm variety have been using in different traditional delicious food item preparations by the indigenous tribes of India (Barman, 2011). However, there is no report on in vitro antioxidant and antigenotoxic property of pupae of the muga silkworm *Antheraea assamensis*. Therefore, the study was design for the first time to evaluate the nutritional potential of pupae of the silkworm *Antheraea assamensis*, based on its chemical composition, antioxidant and antigenotoxic activities.

2. Materials and methods

2.1. Chemicals

DPPH (1, 1-diphenyl-2-picrylhydrazyl), thiobarbituric acid (TBA), catechin, quercetin, tyrosinase (1000 units/ml) were purchased from Sigma, St Louis, MO, U.S.A. Ascorbic acid, sodium dodeycylsulfate (SDS), n-butanol, pyridine, tris–HCl buffer, phosphate buffer, ferrous sulfate, potassium ferricyanide, methanol, Folin–Ciocalteu reagent, dimethyl sulfoxide (DMSO), L-3,4-dihydroxyphenylalanine (DOPA) were purchased from Merck India.

2.2. Sample preparation: preparation of pupae extract

Pupae of the silkworm were purchased from the Udalguri district of Assam and authenticated by an entomologist from Gauhati University, Assam, India. The characteristics of the pupae conformed to the standard criteria. After the sample was washed with distilled water and ground in a meat grinder, it was centrifuged at 3000 rev/min for 20 min to get rid of deposits (mainly pupae shell). The liquid centrifugate was spray-dried and ground into fine powder. The pupae extract were prepared in two solvents: water and methanol. About 10 g of the dried powdered materials were dissolved in 100 ml of solvent for 48 h followed by filtration. The filtrate was then concentrated under reduced pressure using a rotary evaporator (Buchi R-124) at low temperature (<40 °C) for methanolic pupae extract (MPE) and freeze dried for the aqueous pupae extract (APE) and kept in 4 °C until used.

2.3. DPPH assay

The free radical scavenging activity of MPE was determined by the method of Brand-Williams, Cuvelier and Berset (1995). The solution of DPPH in methanol (6×10^{-5} M) was prepared just before UV measurements. Samples were added to DPPH solution in 1:1 ratio followed by vortexing. The reaction was allowed to take place in the dark at room temperature under nitrogen atmosphere. The absorbance at 517 nm was measured at different time intervals. A decreasing intensity of the purple color was taken as increasing scavenging activity. Ascorbic acid served as a standard. The inhibition percentage of radical scavenging activity was calculated using the following equation.

$$\text{Inhibition } (\%) = [(A_0 - A)/A_0] \times 100$$

where A_0 is the absorbance of DPPH in the absence of the sample and A is the absorbance of DPPH in the presence of sample.

2.4. Reducing power assay

Reducing power of MPE was determined according to the method of Oyaizu (1988). 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added to 1 mL sample solution and mixed gently. The mixtures were incubated at 50 °C in a water bath for 20 min. Reaction was stopped by adding 2.5 mL of 10% trichloroacetic acid (TCA) and the mixtures were centrifuged at 4000 rpm for 10 min. From the top layer, 2.5 mL was transferred into tubes containing 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3 \cdot 6\,H_2O$). The resulting solutions were mixed well and after 5 min the absorbance was measured at 700 nm against blanks.

2.5. In vitro lipid peroxidation assay

Lipid peroxidation, induced by Fe^{2+} ascorbate system in rat liver homogenate by the method of Bishayee and Balasubramanian (1971) was estimated as thiobarbituric acid reacting substances (TBARS) by Okhawa, Oohishi, and Yagi (1979). The reaction mixture contained rat liver homogenate 0.25 mL (10% w/v in 0.05 M phosphate buffer, pH 7.4), 0.1 mL Tris–HCl buffer (150 mM, pH 7.2), 0.05 mL ascorbic acid (0.1 mM), 0.05 mL $FeSO_4 \cdot 07H_2O$ (4 mM) and 0.05 mL of MPE of pupae. The mixture was incubated at 37 °C for 1 h and then 1.5 mL 2-thiobarbituric acid (TBA, 0.8% w/v), 1.5 mL acetic acid (20%) and 0.2 mL sodium dodecylsulfate (SDS, 8.1% w/v) were added to the reaction mixture. The mixture was made up to 4.0 mL with distilled water and heated at 95 °C for 60 min. After cooling with tap water, 1.0 mL distilled water and 5.0 mL of mixture of n-butanol and pyridine (15:1 v/v) were added. The mixture was shaken vigorously and centrifuged at 5000*g* for 10 min. After centrifugation, the optical density of the butanol layer was measured at 532 nm in a spectrophotometer.

2.6. Tyrosinase inhibition activity

Tyrosinase activity was determined by spectrophotometry with minor modifications (Masamoto et al., 2003). Tyrosinase from a mushroom solution was prepared at a concentration of 100 units/ml in 0.2 M phosphate buffer solution (pH 6.5). Tyrosinase mushroom solution (400 μL) and phosphate buffer solution at pH 6.5 (300 μL) were mixed with or without the MPE. The mixture was then pre incubated at 25 °C for 5 min before adding 400 μL of 1.25 mM dopa solution, and the reaction was monitored at 475 nm. The percentage of