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In Vitro Evaluation of Antioxidant Activity of Bioproducts Extracted from Silkworm Pupae

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Abstract

Bio products *viz.,* pupal powder, chitosan and pupal oil were extracted from silkworm pupae. The main objective of the present study was to evaluate the antioxidant property of extracted bio products. The activity of antioxidant property was determined with help of 1, 1-diphenyl 1-2-picrylhydroxyl radical (DPPH). Different concentrations ranging from 10-100 µg/ml of silkworm pupal powder were tested for their antioxidant activity. The IC50 value of the pupal powder, chitosan and pupal oil were 60.58 µg/ml, 49.49 µg/ml and 45.89 µg/ml and ascorbic acid showed 51.71 µg/ml, used as a positive scavenger. The scavenging ability of pupal powder, chitosan and pupal oil were 73%, 76% and 64% respectively at 10 µg/ml concentration and standard ascorbic acid showed 64%. The results suggested that the pupal powder and chitosan showed higher scavenging activity and pupal oil and standard ascorbic acid was found to be equally good. Hence, the results confirmed that the bio products obtained from silkworm pupae can be used in food, cosmetics and pharmaceutical industries due to its excellent antioxidant properties.

Introduction

Silkworm pupae are one of the major by-products of reeling industry. It is estimated that 40, 000 metric tons of pupae on dry weight basis (Mahesh *et al.,* 2015) are produced annually which is considered as waste material. Disposal of silkworm pupae is a big problem in silk reeling industries. Silk reelers after reeling out silk used to discard the dead pupae at the outskirts of the city, creating nuisance and health hazards. A dry pupa contains 45-49% of protein (Fagoone, 1983) and 23-24% of oil (Jolly *et al,* 1974), thus forming an important biosource of oil and proteins (Singh and Suryanarayana, 2003). Silkworm pupae find its uses like protein supplement as feed for poultry, swine

and cattle, as manure, moulding material in bakelite industry. The non-defatted silkworm pupae could be used to completely replace fishmeal and could be included up to 50% in the diet. Suresh *et al.* (2012) reported that, the pupae of silkworm are an alternative source of chitin which consequently yields chitosan. Chitosan possesses many beneficially biological properties such as antimicrobial activity (Kobayashi *et al.,* 1990; Tokoro *et al.,* 1989), biocompatibility, biodegradability, haemostatic activity, and woundhealing property, much attention has been paid to its biomedical applications (Farkas, 1990). Furthermore, Owing to these unique properties, chitosan and its derivatives have been proposed for applications in biomedical, food, agriculture, biotechnology and pharmaceutical fields (Felse and Panda, 1999; Kumar, 2000; Shahidi *et al.,* 1999). Tomotake *et al.* (2010) found that, the silkworm pupae possessed n-3 fatty acids, especially α-linolenic acid (36.3%), as a major component. The oil extracted from silkworm pupae is utilized in soap and cosmetic industries. Silkworm pupae oil plays an important role in lowering blood sugar, inhibiting thrombus and regulating blood fat with no liver lipid storage. Additionally it's helpful to regulate inflammatory mediators and interleukins to protect liver. The large amount of fatty acids obtained from pupal oil can be used for the production of biodiesel. Many workers investigated antioxidant properties of commercial chitosan obtained from crustaceans other than silkworm pupae source. However, information on antioxidant property of pupal bio products is not readily available. Hence, the present study was assessed to study the antioxidant activity of pupal products *viz.,* Pupal powder, chitosan and pupaal oil extracted from silkworm pupae.

Materials and Methods

Preparation of silkworm pupal powder

The silkworm pupal powder was dried at 85° C for 4 hours and dried pupae were powdered and used for *in vitro* antioxidant studies.

Extraction of chitosan

The chitin and chitosan extraction involved mainly three steps *viz.,* Deproteinization, Demineralization and Deacetylation. Deproteinization of silkworm pupae were carried out by using 4 per cent dilute sodium hydroxide at 70° C for 4 hours. Silkworm pupae to NaOH ratio of 1:10 (w/v) were maintained. After the treatment, the materials were washed with running tap water for 4-5 times to excess alkali and subsequently rinsed in deionized water. Demineralization of silkworm pupae was carried out by treating 3 per cent Hydrochloric acid at ambient temperature for two hours with deproteinized pupae to liquid ratio of 1:10 (w/ v). The material was washed with running water and rinsed in deionized water. The product obtained was chitin. Chitin was dried in hot air oven for 12h at 50°C for further use. Deacetylation was carried out by treating chitin with 45 per cent concentration of sodium hydroxide at 95° C for 4 hours and the solid to liquid ratio was maintained at 1:12 (w/v). After the treatment, the material was washed with water and rinsed in deionized water. The final product obtained was chitosan. The chitosan was dried in hot air oven for 10 h at 50° C and used for studies (Suresh *et al.,* 2012).

Extraction of pupal oil

The dried and cleaned pupae are first soaked in hexane solvent in a soxhelt apparatus. Material: Liquid ratio of 1: 4 was maintained. After filtering and evaporating to dryness in a vacuum, all oil samples were stored in the dark at 4°C until used (Nipha and Arunyakorn, 1997).

Scavenging ability of pupal products on 1, 1-diphenyl 1-2-picrylhydroxyl radicals (DPPH)

The scavenging effect of chitosan on DPPH radical was examined using the modified method described by Shimada *et al* (1992). The free radical scavenging activity of the pupal products (pupal powder, chitosan and pupal oil) was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method. Different concentrations *viz.,* 10, 20, 40, 60, 80 and 100 µg/ml of pupal products were used for the study. The DPPH solution (0.1mM) in ethanol was made, and 1.0ml of this solution was added to 3.0 ml of extracts solution in water at different concentrations. The mixture was shaken vigorously and left to stand for 30min in the dark and the absorbance was then measured at 517nm against a blank. Ascorbic acid was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation. The mean values were obtained from triplicate experiments.

DPPH of radical scavenging activity (%) = (Control OD-Sample OD / Control OD) × 100

Results and Discussion

DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (Bozin *et al.,* 2008). This assay is known to give reliable information concerning the antioxidant ability of the tested compounds (Huang *et al.,* 2005). Additionally, compared with other methods, the DPPH assay has many advantages, such as good stability, credible sensitivity, simplicity and feasibility. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable nitrogen free radical, to decolorize from purple to yellow colour as the radical is quenched by proton radical scavenger of hydrogen donating antioxidant and subsequently transformed into a non radical form (DPPH-H) (Prior *et al.,* 2005 and Karagözler *et al.,* 2008). The colour changes can be measured quantitatively by spectrophotometer absorbance at 517 nm.

Different concentrations ranging from 10-100 µg/ml of silkworm pupal powder were tested for their antioxidant activity. IC50 value is the concentration of the sample required to achieve 50 % scavenging of the superoxide free radical and it was determined from the plot of % scavenging against concentration. The IC50 value of the pupal powder was 60.58 µg/ml, and ascorbic acid showed 51.71 µg/ml, which is a well-known antioxidant. The silkworm pupal powder was found to influence scavenging activity (Figure 1). The scavenging ability of pupal powder was 73% and ascorbic acid used a

positive scavenger showed 64% at 10 µg/ml concentration. Deori *et al.* (2014) studied the antioxidant activity of pupae of the muga and eri silkworm.

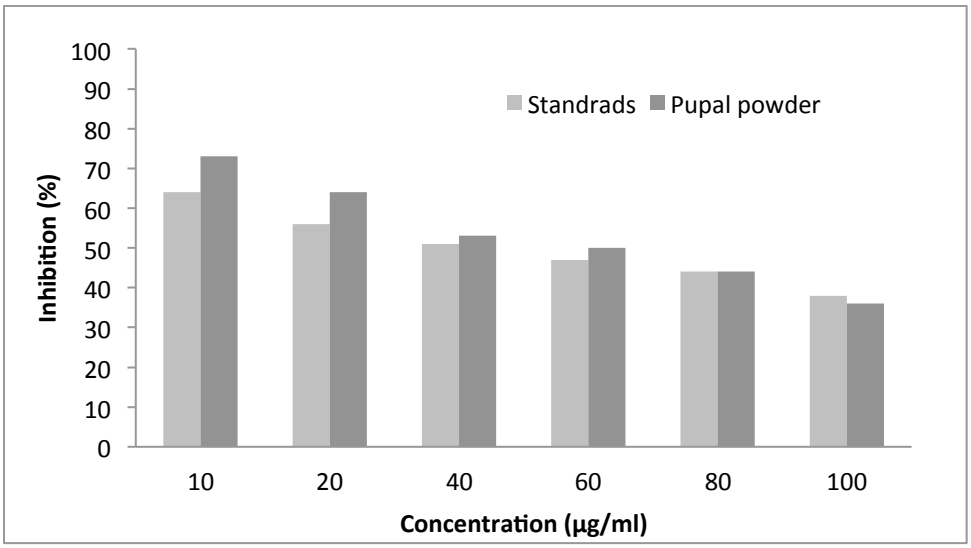

Figure 1 DPPH radical scavenging activity of pupal powder

Chitosan extracted from silkworm was found to possess scavenging activity. The IC50 value of chitosan was 49.49 µg/ml compared to ascorbic acid (51.71 µg/ml). The DPPH radical scavenging potential of chitosan ranged from 24% to 76% at varying concentrations (10-100 µg/ml). The hydroxyl group and amino group are the key functional groups for its antioxidant activity. The scavenging activity of chitosan may be due to the reaction between the free radicals and the residual free amino group (NH2) to form stable macromolecule radicals and/or the amino groups can form ammonium groups (NH3 þ) by absorbing hydrogen ions from the solution and then reacting with radicals through an additional reaction (Xie *et al.,* 2001and Yen *et al.,* 2008). Many researchers have studied on antioxidant properties of chitosan derivatives (Lin and Chou, 2004 and Xing *et al.,* 2005).

Chitosan showed higher scavenging ability of 76% at 10 µg/ml of concentration than standard Ascorbic acid showed only 64%. However, both chitosan and ascorbic acid showed 64% of scavenging ability at 20 µg/ml concentration which indicated that the chitosan was found to be equally good in scavenging activities (Figure 2). Park *et al.* (2004) suggested that chitosan may eliminate various free radicals by the action of nitrogen on the C-2 position of the chitosan. Yen *et al.* (2008) reported that fungal chitosan scavenged DPPH radicals by 28.4-53.5% at 10mg/ml, obviously chitosan from crab shells and *shiitake stipes* was also not an effective scavenger for DPPH radicals. Yen *et al.* (2007) reported the scavenging ability of crab chitosan C60 on DPPH radicals was 28.4% at 10mg/ml, whereas these of other crab chitosan were in the range of 46.4-52.3%. The scavenging ability of chitosan was 38.03% at 1mg/ml. Rajalakshmi *et al.* (2013) extracted chitosan from shrimp exoskeleton showed DPPH radical scavenging activity.

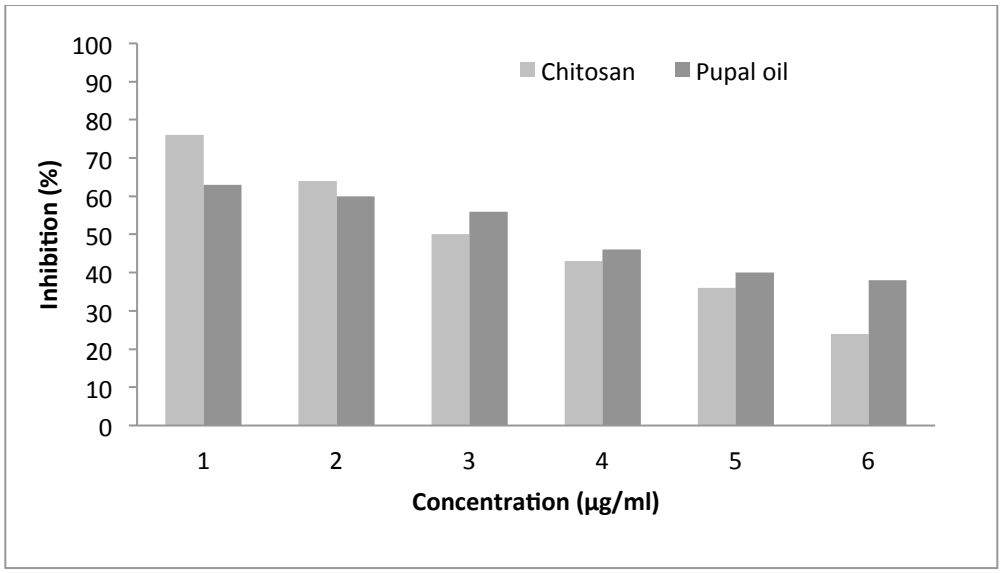

Figure 2 DPPH radical scavenging activity of chitosan and pupal oil

The IC50 value of pupal oil extracted from silkworm pupae was found to be 45.89 µg/ml compared to ascorbic acid (51.71 µg/ml). There is no significant difference between pupal oil and standard ascorbic acid showed 63 and 64% of scavenging activity at 10µg/ml. 20µg/ml and 40 µg/ml of concentration showed scavenging activity of 60 and 56 per cent and 56% and 51% for pupal oil and ascorbic acid (Figure 2). Winitchai *et al.* (2011) studied the free radical scavenging activity of oils extracted from pupae of native Thai silkworm. Pises *et al.* (2006) reported that the silkworm pupae oil contains antioxidant, tocopherol. Also, the oil possesses some fatty acids that was more potent antioxidants (Kotake *et al.,* 2002). Phospholipids and tocopherol existing in pupae oil may also play an important role in protecting the lipids against especially linoleic acid against oxidation (Eiichi *et al.,* 2002) and carotenoids, such as lutein and neoxanthin, and might act as antioxidants in the oils.

Use of synthetic antioxidants is under strict regulation due to their potential hazards. Also, it requires more effort in metabolism than natural antioxidants, which are readily accepted by the body. The results showed that bio products extracted from silkworm pupae were good in antioxidant activity and it could be used as a source of antioxidant in food, cosmetics and pharmaceutical industries. The conversion of pupal by products into high value products in diverse fields adds profitability and provides safe to the environment.

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