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Anti-tyrosinase Activities of Curcumin-Chitosan Gold Nanoparticles Synthesized from Beetle (*Oryctes rhinoceros*)

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A breakthrough in cosmeceuticals by utilizing insects as major ingredients in cosmetic products is gaining popularity. Therefore, the interest in rare sources of ingredients, for instance, from the *Oryctes rhinoceros* beetle, can bring huge benefit in turning pest to wealth. In this study, curcumin was chosen as the active ingredient loaded into chitosan-gold nanoparticles (CCG-NPs). However, curcumin is unstable, has poor absorption, a high rate of metabolism and high sensitivity to light. These are all factors that contribute to the lower bioavailability of any substance to reach the target cells. Therefore, chitosan extracted from *O. rhinoceros* acts as a drug carrier and incorported in gold nanoparticles are used to overcome these problems. The CCG-NPs were successfully synthesized at 70 ºC for 60 min under optimal conditions of reactant ratio of 2:0.5 (0.5 mM $HAuCl_4$:0.1% curcumin). The tyrosinase enzyme inhibition of CCG-NPs from *O. rhinoceros* was 66.385 ± 3.0%. Thus showing a good inhibition trait for the anti-tyrosinase assay as it is almost double the tyrosinase inhibition percentage when being compared to CCG-NPs from the commercial chitosan. Therefore, CCG-NPs from *O. rhinoceros* has a high potential in cosmeceutical applications as whitening agent.

Keywords: Anti-tyrosinase activity, Chitosan nanoparticle, Beetle, Gold nanoparticles, *Oryctes rhinoceros*.

INTRODUCTION

Tyrosinase also known as polyphenol oxidase, is a copper-containing enzyme, which is widely dispersed in nature and involved in melanin formation [1]. It catalyzed the production of quinones and mediated the formation of brown pigment *via* spontaneous polymerization of highly reactive quinones [2]. As a result, inhibiting tyrosinase activity is an important target in the treatment of pigmentation disorders and the development of new whitening agents [3].

Tyrosinase inhibition has long been a goal in skin health research, cosmetics and agriculture due to its role in browning responses in skin pigmentation and during fruit harvesting and handling. To lighten the skin colour, skin whitening and bleaching products use natural or synthetic tyrosinase inhibitors. Tyrosinase inhibitors include polyphenols, benzaldehyde derivatives, long-chain lipids, steroids and natural substances [3]. The anti-tyrosinase activity of curcumin metal complexes as depigmentation agents in cosmetic products was examined by Saewan *et al.* [4], which is of great importance to the cosmeceutical industries.

The term "cosmeceutical" refers to a class of skin care products that combine cosmetic and medicinal functions to enhance both the skin's look and its health. They are applied topically as creams or lotions, similar to cosmetics, but include active ingredients that influence skin cell function. In order to make it function more effectively, the size of cosmeceuticals needs to be nanosized. Nanocosmeceuticals have a variety of advantages. There are many factors that can be used to control how active compounds are released from carriers. These factors include physical or chemical interactions between components, drug composition, polymer and additives, ratio and the process of making them [5].

The cosmetics industry has achieved a breakthrough in the use of insects as vital components in its products in recent years [6,7]. Over the last 50 to 60 years, the cosmetic industry has developed from an era of secret formulations, elusive promises and false optimism to a totally new sector founded on

science. Cosmetics are no longer a stand-alone enterprise [8]. They are becoming more reliant on the cosmetics, pharmaceutical, biochemical and medical industries. As a result, new research productively turns into more potent cure and preventative measures. To achieve the optimum effectiveness and safety, active ingredients in new cosmetic products must be carefully chosen. As a result, novel cosmetics have grown more complex in terms of formulations and presentation, such as medication administration and carrier for improved bioavailability of bioactive chemicals on target cells [9].

Recently, drug carriers that enhance the topical action of active compounds have gotten a lot of attention and chitin is one of them [10,11]. After cellulose, chitin is the second most prevalent natural biopolymer. It is a key component of many fungi's cell walls, insect exoskeletons and crustacean shells. It is mostly made up of waste from the processing of marine foods such as crab, shrimp and krill shells [12]. For commercial chitin production, crustacean sources have been favoured [11]. Therefore, non-conventional chitin and chitosan sources such as corals, fungi and insects might need to be explored to increase industry confidence in using them as alternative to commercial chitin and chitosan.

Since, it is substantial to have an effective cosmeceutical product, incorporating curcumin into cosmetics formulation will certainly turn it into a therapeutic product. Curcumin is an active compound with many benefits. To achieve the goal of cosmeceuticals in dermatology research, it is important to produce new ecologically friendly nanosized compounds for skin nanoparticulate systems. However, curcumin has low intrinsic activity, poor absorption, a high rate of metabolism, inactivity of metabolic products and quick excretion and clearance from the body. These are all factors that contribute to the lower bioavailability of any substance to reach the target cells. Curcumin's bioavailability and biological activity are currently being studied due to its low systemic bioavailability and limited access to certain tissues at sufficient pharmacologic levels *in vivo*. Several delivery strategies are being tested to improve curcumin's bioavailability and biological activity [13].

Chitosan obtained from an insect, specifically *Oryctes rhinoceros*, was used for this study. Insects consume millions of tonnes of plant food each year by chewing, nibbling and sucking it. Insects love all parts of plants, especially the roots, leaves, flowers and seeds. They could even chew their way through wood or bark. Although *Oryctes rhinoceros* is a natural decomposer and regarded as a common invasive insect of coconut in most areas of the world, particularly in Southern Asia [14,15], although it is a serious invasive insect of oil palm (*Elaeis guineesis*) [15]. Because there is a lot of interest in arthropod-derived chitin as an alternative to marine sources, the underutilized chitin from *O. rhinoceros* may help transform pests into money. However, extensive investigations on *O. rhinoceros'* chitin and its derivatives have yet to be completed [15]. Thus, the purpose of this work is to synthesize and characterize the novel mixture of curcumin-chitosan gold nanoparticle from *O. rhinoceros*'s chitin mediated synthesis. The successful accomplishments of this work provide the scientific validation for chitin and chitosan isolated from *O. rhinoceros* as well as the synthesized CCG-NP that has not yet been reported in literature.

EXPERIMENTAL

Preparation of chitosan: For chitin extraction deproteinization was done by strong base (1 M NaOH, 99 ºC, 20 v/w of sample) [16], while decalcification was by strong acid (3.9 M HCl, 75 ºC, 12 v/w of sample) [17] with few modifications. The process continued with deacetylation of chitin where 10 g of chitin was poured in 200 mL of 60 % NaOH, 99 ºC for 60 min. The solution was then filtered and washed until pH 7. The mush was oven dried overnight at 40 ºC. The dried sample was ground using mortar and pastel. Afterwards, the ground sample was sieved using 500 µm siever and kept in 4 ºC airtight container for further use.

Preparation of chitosan-gold nanoparticles (CCG-NPs): The preparation of CCG-NPs was done based on the reported method [18] with few modifications. The CCG-NPs were prepared with a volume ratio of 2:0.5 by adding 2 mL 60 mM $HAuCl_4$ solution to 0.5 mL of 0.1 % curcumin solution in clean bottle covered with aluminium foil to synthesize the curcumin loaded with chitosan into gold nanoparticles, an aqueous solution of TPP (0.1%) was mixed with chitosan solution (3 mL, 0.5 %) while magnetic stirring the solution. After 50 min, an aqueous solution of $HAuCl_4$ (2 mL, 60 mM) was added and the mixture was heated to 70 ºC [19] and stirred for another 45 min [20]. The bottle was left for 24 h in room temperature.

Anti-tyrosinase assay: Anti-tyrosinase activity of CCG-NPs was determined according to the method of Rangkadilok *et al.* [21], with few modifications by Saewan *et al.* [4]. Briefly, 50 mM of phosphate buffer, 5 mM L-DOPA, 2.1 mM ascorbic acid and 0.065 mM EDTA were mixed and equilibrated to 25 ºC. Each different concentration solution of CCG-NPs (10, 20, 40, 60, 80 and 100 µL) from *O. rhinoceros* was added into the mixture The absorbance was monitored using UV-vis at 475 nm until constant reading was achieved for about 10 min. Afterwards, 2500 units/mL of tyrosinase enzyme were added to each respective cuvettes and the reaction started. The absorbance values were recorded immediately at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 at 490 nm. The same method was applied to CCG-NPs prepared from the commercial grade chitosan for comparison. The CCG-NPs with the highest anti-tyrosinase activity was determined for its inhibition mechanism by using a Lineweaver-Burk plot compared with standard kojic acid. The inhibition constant of complex (KI) will be determined by plotting the intercept values *versus* the concentration of the corresponding compound [22]. Kojic acid was used as standard and reference of tyrosinase inhibitor. The percentage of anti-tyrosinase activity, eqn. 1 was applied [4]:

$$\text{Anti-tyrosinase activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where, $\text{Abs}_{\text{control}}$ is absorbance of control at 490 nm, $\text{Abs}_{\text{sample}}$ is absorbance of sample at 490 nm.

RESULTS AND DISCUSSION

The anti-tyrosinase assay measures the rate-limiting enzyme in the melanin production. Thus, inhibiting the tyrosinase enzyme is useful in managing hyperpigmentation and related disorders [22]. Melasma and ephilids are the skin darkening conditions that have been linked to melanin overproduction [3]. Tyrosinase is a copper-containing enzyme found in plant and animal tissues that catalyzes the oxidation of tyrosine to produce melanin and other prigments. It is located inside the melanosomes, which are produced by skin melanocytes [22].

Anti-tyrosinase activity: Tyrosinase inhibitory activity was determined using spectrophotometric method. Tyrosinase activity was determined *via* L-DOPA as substrate. Tyrosinase supposedly catalyzed the conversion of a phenolic substrate to a quinone intermediate, which reacted with tyrosine enhancer forming a highly stable chromophore with absorbance at 510 nm. In this study, CCG-NPs acted as tyrosinase inhibitors and worked by blocking or sedating the production of melanin, which causes your skin to have dark spots. Therefore, it has the potential as whitening agent.

This assay was done in volume instead of concentration of CCG-NPs. Apparently, the CCG-NPs are solid with a metal bond (metals) or a covalent bond (oxides). Such nanoparticles do not dissolve in common organic solvents and even water [23]. Therefore, CCG-NPs was unable to redissolve once dried.

In this study, 2500 units/mL of tyrosinase enzyme was used for anti-tyrosinase assay of CCG-NPs. The sample C2 represented CCG-NPs synthesized from *O. rhinoceros'* beetle, sample C10 represented CCG-NPs with commercial grade chitosan while sample C16 represented a blank CCG-NP (no chitosan). Fig. 1 showed that sample C2 exhibited the highest inhibition percentage followed by samples C10 and C16. Kojic acid was used as standard reference as well as a positive control while sample C16 acted as negative control. The inhibition percentage for sample C2 started slowly from 32.566 ± 1.03% when the volume was 10 µL and gradually increased to 66.385 ± 3.00% (100 µL). For sample C10, the inhibition percentage started at 31.769 ± 2.03% at 10 µL and continued to rise slightly to 38.604 ± 0.94 % at 10 µL. The lowest tyrosinase inhibition rate was exhibited by sample C16, when the volume of sample was 10 µL, the inhibition percentage was 20.813 ± 1.65% and reached maximum at 30.015 ± 1.64%, when the volume increases to 100 µL.

Previous results [24] stated that curcumin at the concentrations of 0.2 and 2 µg/mL inhibited tyrosinase activity by 19.4% and 21.8%, respectively. This inhibition percentage showed a much lower value as compared to samples C2 and C10. Furthermore, without chitosan, the mixture also exhibited similar value to sample C16 (20.81%). Hence, this can prove that chitosan acts as biocarrier and stabilized curcumin to work better in inhibiting tyrosinase enzyme [25].

The results indicated that the tyrosinase inhibitory effects of CCG-NPs was due to the reduced size of nanoparticles and thus improved bioavailability and proposed emulsification as a successful delivering method for curcumin. Hence, CCG-NPs showed a good potential as tyrosinase enzyme inhibitor therefore can be a potent whitening agent.

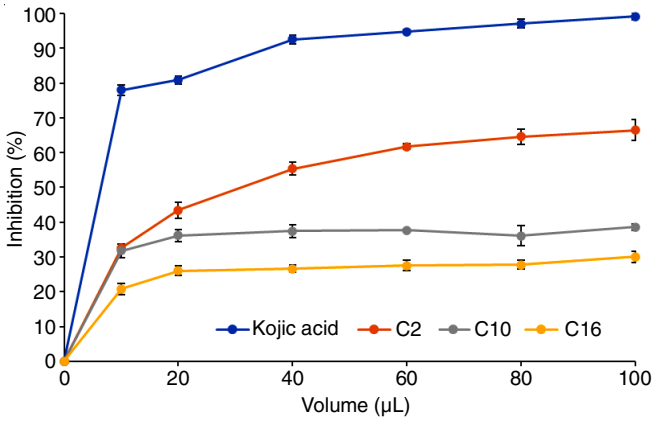

Fig. 1. The inhibition % profile of anti-tyrosinase enzyme assay using kojic acid as positive control and sample C16 as negative control

Conclusion

The chitosan obtained from *Oryctes rhinoceros* beetle exhibited a good inhibition trait for the anti-tyrosinase assay and almost double effective as compared to the commercial chitosan. Although CCG-NPs with *O. rhinoceros* chitosan still cannot reach the same inhibition percentage as kojic acid, it can still be a good natural alternative as a whitening agent. Uniform skin tone is very desirable and by having tyrosinase inhibition properties, depigmentation and lightening of spots can be achieved. Therefore, CCG-NPs has very high potential in cosmeceutical applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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