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Original Research Paper

Development and assessment of tyrosinase inhibitory activity of liposomes of *Asparagus racemosus* extracts

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ABSTRACT

The purpose of this study was to develop liposomal formulations of *Asparagus racemosus* root extract (AR1-6) as well as evaluate the physicochemical properties and *in vitro* tyrosinase inhibitory activity. Liposomes composed of AR1-6 to lipid weight ratio of 1:10 and lecithin (LEC) or Phospholipon® 90G (PC90G) as structural phospholipid at 7:3 molar ratio to CHOL were prepared by various methods, i.e. chloroform-film (CF), reverse-phase evaporation (REV), polyol dilution (PD), and freeze-drying of monophase solution (MFD) methods. The results revealed that vesicles prepared by CF and MFD were multilamellar whereas those prepared by REV and PD were oligolamellar in nature with particle sizes ranging from 0.26 to 13.83 μm. The zeta potentials were in the range of −1.5 to −39.3 mV. AR1-6 liposomes with LEC possessed significantly higher entrapment than those with PC90G. The highest entrapment efficiency and *in vitro* tyrosinase inhibitory activity of 69.08% and 25%, respectively, were obtained from liposomes having LEC and prepared by PD method. The tyrosinase inhibitory activity were in the rank order of LEC > PC90G, and PD > CF > REV > MFD. It could be concluded that the mechanism of vesicle forming in each method of preparation was the key factor influencing physicochemical properties, particularly vesicle type, size, surface charge, and entrapment, which were well correlated with the biological activity.

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1. Introduction

The increase in production and accumulation of melanin are the cause of a large number of skin ailments, e.g. acquired hyperpigmentation such as melisma, postinflammatory melanoderma, solar lentigo, etc. The hyperpigmentation of the epidermis and dermis depend on either increased numbers of melanocytes or the activity of the enzyme [1]. The melanocytes are responsible for the biosynthesis of melanin through enzymatic conversion of L-tyrosine which leads to the suntanning effect. However, overproduction of melanin pigment is not desirable, especially on people's face [2,3].

The plant *Asparagus racemosus* Willd. (Liliaceae) is commonly called, in India, Satavari; Satawar or Satmuli in Hindi; Satavari in Sanskrit; Shatamuli in Bengali, etc. In Thailand it is called Sam-Sib or Sam-Roi-Rak. The plant is a spinous under-shrub, with tuberous, short rootstock bearing numerous succulent tuberous roots (30–100 cm long and 1–2 cm thick), which are silvery white or ash colored externally and white internally [4]. Pharmacological studies with animals have manifested the potency of *A. racemosus* extract as an antioxidant, anticancer, anti-inflammatory, anti-aging, and as a substrate of inulinase production for ability in modulating the immune system [5]. Moreover, the tyrosinase inhibitory activity as skin whitening effect has also been found from the investigation of this extract by using dopachrome microplate assay [6,7]. The root extracts of *A. racemosus* have been employed in two major forms as methanolic and aqueous extracts, the products of which were in the form of tablets and syrup [4]. It has been reported that the major active constituents of *A. racemosus* are steroidal saponins (Shatavarins I–IV) present in the roots [4]. Besides, other active compounds such as quercetin, rutin, kaempferol, racemofuran, and isoflavone were also found and identified on the basis of chemical and spectroscopic evidence. It has previously been observed that a number of flavonoids were found to manifest the tyrosinase inhibitory activity with some flavonols acting as copper chelators [29]. Besides, quercetin and kaempferol were found to inhibit the oxidation of L-DOPA catalyzed by mushroom tyrosinase. As a result, *A. racemosus* extract might be a candidate for therapeutic applications in preventing pigmentation disorders and other melanin-related health problems as well as for cosmetic applications for skin whitening effect.

Liposomes have long been receiving a lot of attention during the past thirty years as drug targeting systems of great potential. More recently, many new developments have been emerging in the area of liposomal drugs, from clinically approved products to new experimental applications, with gene delivery and cancer therapy still being the principal areas of interest [8]. The pharmaceutical and pharmacological justifications of the use of liposomes as drug carriers are well recognized [9]. Numerous liposomal drugs have been approved for undergoing clinical evaluation, e.g. cytarabine, vincristine, lurtotecan, platinum compounds, DNA plasmid encoding HLA-B7 and α2 microglobulin, all-*trans* retinoic acid, and *E1A* gene [8].

In this study, liposomes of *A. racemosus* extract for therapeutic applications in preventing pigmentation disorders were prepared by various methods, i.e. chloroform-film, reverse-phase evaporation, polyol dilution, and freeze-drying of monophase solutions methods, their physicochemical properties and tyrosinase inhibitory activities of which were evaluated.

2. Materials and methods

2.1. Materials

Phosphatidylcholine (PC90G) (Phospholipon® 90G) was a gift from Rhône Poulenc Rorer, Köln, Germany. Lecithin (LEC) was purchased from Union Chemicals, Bangkok, Thailand. Cholesterol (CHOL) and *tert*-butanol (TBN) were obtained from Carlo Erba Reagents, Rodano, Italy. Diosgenin (DG) was obtained from Sigma–Aldrich®, St. Louis, MO, USA. Kojic acid, 3,4-dihydroxy-L-phenylalanine (L-DOPA), mushroom tyrosinase, sucrose (SUC), and vanillin were purchased from Sigma–Aldrich®, Steinheim, Germany. Chloroform, methanol, and sulfuric acid, 96% (v/v), were from Labscan, Stillorgan, Co Dublin, Ireland. Dibasic potassium phosphate, monobasic potassium phosphate, and sodium hydroxide were from Ajax® Finechem, Auckland, New Zealand. Absolute ethanol was from Merck KGaA, Darmstadt, Germany.

2.2. Plant materials

The roots of *A. racemosus* were collected from Tak Province, Thailand. The plants were identified by the botanical staff and the voucher specimen of the plant was deposited in the herbarium of Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand.

2.3. Extraction of A. racemosus roots (AR1-6)

A. racemosus roots were dried and minced into powder and then extracted by maceration with 95% ethanol at 25 °C for 24 h. The supernatant was filtrated and evaporated by a rotary evaporator (Eyela® A-3S, Tokyo Rikakikai, Japan). The dark brown viscous liquid obtained thereafter was freeze-dried (Christ® LOC-1m, Göttingen, Germany), and kept at 4 °C until use.

2.4. Thin layer chromatography (TLC) fingerprint

TLC was performed on silica gel plate, $60F_{254}$ (Merck KG, Darmstadt, Germany) and the developing system of 2:1-hexane:ethyl acetate was used in this study [10]. Solution of DG (0.5 mg/ml) in methanol was used as reference [11,12]. The sample solution of AR1-6 (10 mg/ml) was prepared by dissolving in methanol and then filtering through 0.2-μm nylon membrane filter before use. DG solution and AR1-6 sample at 8 μl were spotted by Autospot equipped with scanner and camera (Camag®Limonat V, Switzerland) on the TLC plate, which were then air-dried for 5 min and run with the developing system. After running was complete, the plate was dried in the oven at 100–110 °C for 5 min, sprayed with 10 ml anisaldehyde-sulfuric acid reagent, and then heated at 105 °C for 5–10 min. Afterward, the plate was visualized under visible (366 nm) and UV light (254 nm) (Perkin Elmer® Lambda 35, Waltham, Massachusetts, USA) and TLC fingerprints were photographed. This experiment was performed in duplicate.