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Studies on Natural Cosmetic R & D – From Laboratory to Prototype Product§

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Abstract

Cosmetic sector has been developing day by day and more products based on scientific search are released to the market. In this sector, particularly natural cosmetics are in a great demand, which also raises the need for more effective and safe products. Being one of the most important biodiversity spots in the world due to its rich flora, it must be a target for Turkey's national economy to use plant species in cosmetic preparation as well as branding for our country's promotion. For this purpose, we have been working on R&D of natural cosmetics using our own herbal sources to produce cosmetics with anti-wrinkle and skin bleaching effect. In this regard, we started to screen over 100 plant species for their anti-wrinkle and skin bleaching potential in our laboratory by enzyme inhibitory assays against tyrosinase, elastase, and collagenase using ELISA microtiter methods. The active extracts were subjected to activity-guided fractionation and isolation and then, compounds responsible for the aforementioned inhibitions were elucidated. The active inhibitory molecules were proceeded to *in silico* assays using molecular docking techniques. According to our preliminary screening results, the extracts from *Vitis vinifera*, *Camellia sinensis*, *Cotynus coggygria*, *Maclura pomifera*, and *Geranium glaberrimum* were found to be the most active extracts, from which we prepared several cosmetic formulations under the brand name Vitabel. In this paper, summary of our extensive studies on cosmetic R & D conducted in our laboratory which were finalized with prototype cosmetic samples. Our findings indicate that plants are notable biosources for cosmetic research.

Key words: Natural cosmetics, anti-wrinkle, skin bleaching, R&D, plants

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1. Introduction

Cosmetic industry has been fast developing worldwide depending on the need from consumers. Within this industry, natural cosmetics with premium quality are even more demanded, although many renowned brands are available in the market. Green theme has become so popular for the market for natural cosmetics since environmentally-friendly and safe cosmetics are prevalent. Besides, a shift from basic products to more advanced products is observed. In this regard, relatively new concept called "cosmeceuticals" (drug-like cosmetics) has emerged, which is formed as combination of "cosmetics" and "pharmaceuticals". Cosmeceuticals are described as "topical cosmetic-pharmaceutical hybrids used to maintain and improve appearance and beauty of the skin". For example; a shampoo is a cosmetic because its intended use is to clean the hair,

whereas an antidandruff shampoo is a drug because its intended use is to treat dandruff. Although they first appeared in the world market in 1996 (Draelos, 1997), the first person to use the term "cosmeceuticals" was Kilgman (Kilgman, 2005). However, drug-like effects of cosmeceuticals which target dermatological tissues seem to be largely unproven, yet. In this scope, another modern term has also emerged as "nutricosmetics" or "oral cosmetics". Although there is no legal category called cosmeceuticals, the term has found application to designate the products at the borderline between cosmetics and pharmaceuticals (Lee, 2016).

The principal product lines of cosmeceuticals are anti-aging, while only a very few of them are anti-acne or moisturizers (Baumann, 2007). On the other hand, it is clear that many of anti-aging cosmeceuticals have been formulated with unconfirmed clinical claims. Therefore, claimed effects should be proven on scientific platform. Besides, safety is also a critical topic required for cosmeceuticals considering consumer health. Actually, the following points must be tested for cosmetics including benefit perceived by consumers; safety of formulation, evidence for marketing claims as well as use ıf natural and sustainable sources (Draelos, 2014).

Taking these points for cosmetics into consideration, we have started an extensive study approximately 8 years ago to develop new antiaging cosmetic formulations in our laboratory. This paper will describe summary of our studies on natural cosmetics since the year of 2010.

2. Results and discussion on our studies on natural cosmetic formulations

Since the year of 2000, I have been working on enzyme inhibitory potential of natural sources including plants and marine organisms through screening them against cholinesterase family (acetylcholinesterase and butyrylcholinesterase), tyrosinase, lipoxygenase, xanthine oxidase as well as some other enzymes (phosphodiesterase-I, carbonic anhydrase-II, urease, etc) in collaboration with other research groups. In addition, as I was very interested in, we began to work on elastase and collagenase, the enzymes related to cosmetic research for skin-aging purposes. In this regard, we initially screened over 100 extracts from numerous plants. By the way, our initial screening relevant to cosmetic research provided us with "3rd Prize in the Category of Cosmetics, Soap and Cleansing Products" at 2nd R&D Project Fair organized by Istanbul Chemicals and Chemical Products Exporters' Association in 2012, which motivated our group even more to go further in this scope.

Our criteria to select the plant species to screen for these targets was as follows;
Usage in traditional medicine especially for skin diseases,
High phenolic content,
Constituent of a cosmetic product,
Similar studies in different countries

Therefore, the ethanol extracts were prepared and tested against tyrosinase, elastase, and collagenase using in-house methods that are established by our group in our laboratory. The initial screening of the plant extracts led to identification many effective inhibitors (unpublished data). Among them, *Cotinus coggyria*, *Tripleurospermum oreades*, *Garcinia mangostana*, *Lamium purpureum* subsp. *purpureum*, *Maclura pomifera*, *Crataegus monogyna* subsp. *monogyna*, and *Camellia sinensis* (green tea) extracts were found to have over 50% inhibition against tyrosinase, while *Cotinus coggyria*, *Pistacia vera*, *Citrus aurantium*, and *Vitis vinifera* were active towards elastase. The extracts with effective inhibition against collagenase were elucidated as *Cotinus*

coggyria, *Garcinia mangostana*, *Punica granatum*, and *Vitis vinifera*. Among them, we selected *Cotinus coggyria* to study further by activity-guided fractionation and developing prototype cosmetic formulations. The extensive and detailed chromatographic studies on *Cotinus coggyria* extract led us to identify several phenolics (*e.g.* quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, gallic acid methyl ester, etc) in the active fraction. After that, we prepared two nanoemulsions separately from *Vitis vinifera* and *Camellia sinensis* (green tea) extracts for anti-wrinkle purpose, while two cream formulations were prepared from the extracts of *Cotinus coggyria* and *Maclura pomifera*. These prototype products were prepared under the brand name Vitabel created by our group. In fact, this part of our work was selected in the best 10 projects among 359 projects in total for the competition of women in innovation organized by TUBITAK-Marmara Teknocity & Society of Woman & Democracy (KADEM) in 2015.

On the other hand, stability tests were conducted in collaboration with Vivatinell Cosmeceuticals (London, UK). Particle size measurements for our nanoemulsions were carried out at Department of Pharmaceutical Technology in our Faculty (unpublished data). Later on, we prepared another cream formulation using the extract of *Citrus maxima* peels, which brought us an R&D award called "*Citrus special award*" presented by Aegean Exporters' Associations at 4th International Food R&D Brokerage Event in 2016.

In our ongoing studies, we have lately focused much more on skin-whitening cosmetics. That kind of products generally contain hydroquinone, arbutin, ascorbic acid (vitamin C), vitamin E, alpha-kojic acid, azelaic acid, tretionine, glabridine, etc. as depigmenting agents (O'Donoghue, 2006; Pillaiyar *et al.*, 2017). Nevertheless, some of them possess severe side effects and even banned to use in cosmetics due to their toxicity on skin (Desmedt *et al.*, 2014 & 2016; Matsumoto *et al.*, 2016). Thus, safer skin-whitening agents are still needed by consumers. Based on this information, we performed a large screening on synthetic derivatives of alpha-kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one), a skin whitening natural metabolite isolated firstly from a microfungus, *e.g. Aspergillus flavus*, in collaboration with Faculty of Pharmacy, Hacettepe University (Ankara, Turkey). For this purpose, over 100 derivatives were tested and two of them were patented by Turkish Patent Organization in 2017 and US Patent Office in 2018. The work performed by our team was awarded with "*silver medal*" at International Scientific Invention Fair by Turkish Ministry of Science and Technology in 2017 as well as "*best academic invention medal*" by International Federation of Inventory Associations (IFIA) in 2018.

Cinnamic acid, a phenylpropanoid derivative natural compound found widely in plant kingdom, has been reported to have inhibitory effect on melanin biosynthesis. In continuation of cosmetic-related research, we also tested a number of cinnamic acid derivatives (based on ferulic acid skeleton) for its tyrosinase inhibitory potential (Gür *et al.*, 2019). When we tested piperazine amides of chemically synthesized cinnamic acid derivatives, several derivatives exerted promising tyrosinase inhibitory effect displayed by both *in vitro* and *in silico* experiments. Molecular docking simulations into the active site of *Agaricus bisporus* mushroom tyrosinase have been carried on these compounds in order to define the binding interactions. Besides two ferulic acid-piperazine hybrids (IC_{50} = 66.5 μM for 9 and 61.1 μM for 11) and a *p*-coumaric acid-piperazine hybrid (17, IC_{50} = 66.5 μM) were elucidated to be the most potent inhibitors as compared to that of the reference (kojic acid, IC_{50} of 48.3 μM). In a previous similar study by our group, we had reported a strong tyrosinase inhibitory activity by *in vitro* and *in silico* approaches

(Senol *et al.*, 2014). Our findings on *in silico*-based experiments *via* molecular docking calculations and scoring, docking search algorithm, and data plotting revealed that ascorbic acid is a strong inhibitor of tyrosinase through interactions with four amino acid units (histidine 263, serine 282, phenylalanine 264, and valine 283) in the active site of the enzyme. In addition, the compound was also observed to have two long distant hydrogen bindings with Cu1 and Cu2 with distances of 3.57 and 3.41 Å, respectively, through its O5 atom.

More recently, we have also screened all *Geranium* and *Erodium* species from Geraniaceae family growing in Turkey for their tyrosinase inhibitory potential and, among them, tyrosinase inhibitory activity of *Geranium glaberrimum*, a narrow endemic plant species to Turkey, was further investigated using advanced chromatographic methods. LC-Q-ToF analysis of the active fractions pointed out to presence of several phenolic compounds such as ellagic acid, geraniin, corilagin, gallic acid, quercetin, etc. (unpublished data). These compounds were subjected to molecular docking simulations, which directed to existence of strong aromatic pi-pi interactions as well as hydrogen bindings and interactions with copper atoms located in the center of the enzyme. Using these data, a prototype product containing a skin-whitening formulation from *Geranium glaberrimum* extract was prepared in collaboration with MacPharma (Ankara, Turkey).

3. Conclusion

An immense amount of research has so far presented that plants are abundant source of bioactive metabolites which can be used for human health care. Besides, their phytochemical richness and consumer demand led to their use in cosmetics as active ingredients. Despite of a big demand in the world for natural cosmetics, a lot plant species have still remained unexplored from the cosmetic research point of view. Our aforementioned studies on R&D of natural cosmetics have underlined once more their irrevocable importance in this area.

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