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Review

Plant Cell Cultures as Source of Cosmetic Active Ingredients

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Abstract: The last decades witnessed a great demand of natural remedies. As a result, medicinal plants have been increasingly cultivated on a commercial scale, but the yield, the productive quality and the safety have not always been satisfactory. Plant cell cultures provide useful alternatives for the production of active ingredients for biomedical and cosmetic uses, since they represent standardized, contaminant-free and biosustainable systems, which allow the production of desired compounds on an industrial scale. Moreover, thanks to their totipotency, plant cells grown as liquid suspension cultures can be used as "biofactories" for the production of commercially interesting secondary metabolites, which are in many cases synthesized in low amounts in plant tissues and differentially distributed in the plant organs, such as roots, leaves, flowers or fruits. Although it is very widespread in the pharmaceutical industry, plant cell culture technology is not yet very common in the cosmetic field. The aim of the present review is to focus on the successful research accomplishments in the development of plant cell cultures for the production of active ingredients for cosmetic applications.

Keywords: plant cell cultures; active ingredients; cosmetics

1. Introduction

Plants are unique organisms able to create their own food through photosynthesis and provide oxygen to the atmosphere. They also are important sources of food and therapeutic compounds for all

other living organisms. Plants are notable for their high synthetic versatility: the spectrum of chemical structures synthesized by the plant kingdom is broader than that of any other group of organisms, which makes the plants the biggest source of natural remedies in the fields of pharmacy, food and cosmetics [1]. In fact, humans have developed a broad knowledge of useful plants over time through continuous contact with their natural environment, and the use of plants and plant extracts in traditional medicine has been known since ancient times.

Due to a large demand of natural compounds by a constantly growing world population, commercially interesting plants are increasingly cultivated on an industrial scale; but in many cases the cropping techniques and the extraction methods have not been optimized and, as a consequence, the whole process turned out to be economically unsustainable. Plant cell cultures certainly represent a valid alternative for the production of cosmetic active ingredients, since they always provide standardized, contaminant-free and biosustainable products, whose production can be easily extended to an industrial scale.

Thanks to more recent advances in the research on plant physiology and cell biology, huge steps forward have been made in "plant tissue culturing" techniques, which have taught how to handle and manipulate plant cells in the laboratory under sterile and controlled conditions.

The two characteristics that most distinguish plants among all living organisms and are fundamental to understand plant tissue culturing are plasticity and totipotency. Plasticity is the capacity of the plants to change their metabolism, adapting their growth and development to the surrounding environmental conditions. In nature, plant cells are able to initiate cell division from almost any tissue, to regenerate and to switch on different biosynthetic and developmental pathways according to stress conditions. *In vitro* plant cells have very high levels of plasticity as well, and are able to regenerate tissues, organs and even entire plants trough a process of cell dedifferentiation and subsequent differentiation. Moreover, a certain amount of undifferentiated totipotent cells are always maintained in the meristems located in the tips of shoots and roots or inside the vascular system, allowing fast cell division under proper stimuli [2]. For this reason, even though scientifically correct, it is generally redundant to use the term "stem" when talking about plants, since the totipotency is an intrinsic feature of each differentiated plant tissue belonging to adult plants and not only to embryonic developmental stages.

2. Plant Tissue Culture Techniques

Although it is very widespread in the pharmaceutical industry, plant tissue culturing is not yet common in the cosmetic field; more established are single compounds synthesized by or extracted from microorganisms such as bacteria, yeasts or microalgae [3].

Plant tissue culturing allows the propagation of undifferentiated plant cells either to regenerate a whole plant or to produce single cells in culture for further production of secondary metabolites [4]. The plant tissue material that is used to initiate a plant cell culture is called explant. As explants, all plant tissues could be used with different efficiency depending on the plant species. As a result of wounding, the cells on the surface of the explant expand their volume, start to divide, dedifferentiate and form a mass of cells called callus. The callus could be maintained *in vitro* practically for unlimited time using the correct growth medium. In practical terms though, identifying the culture conditions and stimuli required to manifest this totipotency can be extremely difficult and often entirely depends on the responsiveness of a certain plant species, thus it can be largely an empirical process.

When callus cells are cultured in liquid medium, they form a fast growing suspension culture of single cells or small clusters of cells [4]. Plant cell cultures grown in liquid media can now be upscaled to much bigger volumes and employed for the production of commercially interesting compounds for industrial applications, including cosmetics. There are several advantages of using plant cell cultures as sources of active ingredients respective to plants grown in the field: (i) continuous supply of fresh material, independently of the seasons or the plant reproductive cycle; (ii) the growing conditions can be easily standardized in order to always have high levels of consistency from batch to batch; (iii) the extracted components are safe and clean, which means that there is no risk of pathogens or environmental contamination; (iv) the production system is highly sustainable: no agricultural land is needed, meaning less water consumption and less waste material; (v) versatility, as the cultures can be elicited by using genetic or biochemical tools: the concentration of desired compounds can be increased and optimized by changing the culturing conditions, physical parameters or by adding an inducing compound to the culture media; (vi) in most of the cases, the extraction process is easier and less time-consuming. Moreover, if a recombinant protein has to be expressed in a cell culture system, it can be tagged by genetic engineering and easily isolated by single step chromatography, with no need of complex purification steps and fractioning. For all the advantages explained, different types of cosmetic active ingredients have been developed and produced from plant cells grown as liquid suspension cultures; some of the most representative examples are described in the next paragraph. A summary of the main activities of the plant cell culture extracts described below and their main effects on gene expression in skin cells are reported in Table 1.

3. Plant Cell Culture Derived Cosmetic Ingredients

It is sometimes thought that entire plant cells are added to the cosmetic product and kept alive, but that cannot be true. It is impossible to maintain live plant cells in a cosmetic formulation due to the absence of specific growth media, the right osmotic conditions, or the presence of preservatives [5]. Plant cells are indeed an excellent source of active compounds and thus can be used to obtain different types of extracts that are then included in cosmetic formulations. As known, 55% of the plant cell volume consists of cell wall and membranes. The rest is cytosol (the intra-cellular fluid that is present within the cell) where different organelles are positioned, and that consists of 90% water. During the life cycle, plants produce various metabolites with different chemical natures (for example, oil-soluble or water-soluble), which are stored in different cell compartments. That is why the preparation of the plant cell extracts is critical in order to obtain efficient active ingredients that are rich in specific desired compounds. It is always possible to obtain more than one active ingredient from the same culture by using different extraction procedures and solvents, and taking advantage of the chemical nature of plant cell constituents.

3.1. Hydrosoluble and Liposoluble Extracts

One example of how water soluble (hydrosoluble) and oil-soluble (liposoluble) extracts may derive from the same plant cell culture is provided by wild red raspberry (*Rubus ideaus*) [6]. *Rubus idaeus* plants contain several water soluble compounds, such as amino acids, glucides, oligoelements and different classes of polyphenols (flavonoids, anthocyanins, and phenolic acids), which have high

antioxidant capacity and considerable therapeutic properties in protecting skin cells from damages induced by oxidative stress [7]. The species of *Rubus* are rich in liposoluble compounds as well, such as vitamins, tocopherols and fatty acids, in particular linoleic, palmitic and stearic acids [8]. The hydrosoluble cell culture extract of *Rubus ideaus* was shown to exhibit high anti-inflammatory activity (significantly decreasing the expression of the inducible Nitric Oxide Synthase 2 (iNOS2) and the Cyclo-oxygenase 2 (COX2)), mainly due to the presence of high levels of flavonoids and anthocyanins. In addition, the extract provided strong antioxidant power due to the presence of natural phenolic components and induced the genes responsible for DNA protection and repair, such as the gene of the Growth Arrest And DNA Damage-Inducible Protein 45 alpha (GADD45α) and that of the Sirtuin-1 (SIRT1); the effect was even higher than that produced by resveratrol, well known for its capacity to increase cell longevity by activating molecular mechanisms of protection (Table 1) [6]. In contrast, the liposoluble extract obtained from *R. ideaus* cell suspension cultures was able to induce the expression of the most important genes involved in skin hydration and moisturization, including hyaluronic acid, suggesting an equivalent important role as a cosmetic active ingredient and its hydrosoluble counterpart (article in preparation).

Table 1. Activities of the plant cell culture extracts and their main effects on gene expression in skin cells.

Type of extract	Gene expression in skin cells	Main activity in skin cells	References
Rubus ideaus hydrosoluble extract	iNOS2 and COX2 down-regulation GADD45α and SIRT1induction	Anti-inflammatory activity DNA protection and repair	[6]
Malus domesticus whole lysate	Cyclin B1, Cyclin E1 induction	Reversion of senescence signs	[9]
Nicotiana sylvestris cell wall preparation	GAD45α, SIRT-1 and SIRT-6 induction COL I and COL III induction; MMP1, MMP3 and MMP9 down-regulation	DNA protection and repair Collagen synthesis and protection	[10]
Lycopersicon esculentum hydrosoluble extract	COL I and COL III induction; MMP1, MMP3 and MMP9 down-regulation GADD45α and SIRT1 induction	Collagen synthesis and protection DNA protection and repair	[11]
Coffea bengalensis hydrosoluble extract	INV, FLG, AQP3 induction COL I and COL III induction	Epidermal hydration Collagen synthesis	[12]
Verbascoside in *Syringa vulgaris/Buddleja davidii* cell cultures	–	Antioxidant, anti-inflammatory activities	[13,14]
Trans-resveratrol in *Vitis vinifera* cell cultures	–	Antioxidant activity	[15]
Vitis vinifera LME and LCE mixture	COLI and HAS3 induction; Proteasome stimulation	Hydration and cell detoxifying activity	[16]
Dolichos biflorus hydrosoluble extract	iNOS2, COX2, IL1β, IL6 and IL8 down-regulation; DNA repair mechanism activation	Anti-inflammatory activity and UV damage protection	[17]
Paclitaxel in *Taxus cuspidate* cambial meristematic cell cultures	–	UV protection and anti-cancer properties	[18]

In order to obtain a single extract, containing both hydrosoluble and liposoluble compounds produced by plant cells, an apple (*Malus domesticus*) cell suspension culture was developed in disposable, middle-scale bioreactors [9]. A whole lysate of the culture was prepared using high pressure homogenization, resulting in the destruction of the cell membranes to set the extractable ingredients free and generating finely dispersed liposomes (nanosomes) which contained the fat soluble ingredients of the cells. Further, with the application of this extract it was possible to prove the effect of maintenance and protection against UV radiation of the human stem cells and the reversion of senescence signs in fibroblasts [9].

3.2. Plant Cell Wall Derived Active Ingredients

Additional types of extracts were obtained by using plant cell suspension cultures, such as mixtures of peptides and sugars, prepared by hydrolyzing cell wall glycoproteins [10], thus increasing the number of potential cell-culture derived products on the skin care market.

The idea of employing plant-derived small peptides and amino acid derivatives in skin care was born from the assumption that certain classes of peptides were able to induce analogous defense response mechanisms both in plants and animal cells. In plants they increased the tolerance to a wide range of abiotic stresses [19] and in human skin cells they activated specific signaling pathways leading to up-regulation of anti-aging genes [20]. This is particularly true for peptides rich in proline and hydroxyproline, which are the amino acids mostly involved in the induction of defense response mechanisms in living organisms. The cell walls of cultured plant cells are particularly rich in glycoproteins, which are induced during the de-differentiation process that occurs when pieces of plant tissue are put in cultures and calluses are formed. The response of the tissue that has been put into a culture is akin to a wound response, which is known to be accompanied by the enhanced expression of cell wall proteins, having high proline and hydroxyproline content [21,22].

Most of the peptides and amino acid mixtures used as ingredients in cosmetics and the food industry contain either chemically synthesized proteins or peptides obtained by partial digestion of animal proteins like collagen and elastin, which involves certain risks associated with the animal-derived products.

A plant cell wall preparation obtained from woodland tobacco (*Nicotiana sylvestris*) cell cultures showed strong anti-ageing properties when tested on skin cells, since it was particularly rich in amino acids like glycine, proline and hydroxyproline, the most abundant in mammalian collagen [10]. Besides the presence of peptides, the preparation also contained sugars derived from the digestion of cell wall glycoproteins. Although the role of sugars in cosmetics has not been completely defined yet, it was shown that saccharides contained in a plant extract could have beneficial effect on hydration and an anti-inflammatory effect on dermal cells [23].

The mixture of peptides and sugars obtained from the digestion of cell wall glycoproteins of *Nicotiana sylvestris* cell cultures revealed interesting protective effects against oxidative stress and multiple activity in up-regulating the genes of GAD45α, SIRT-1 and SIRT-6 involved in DNA protection and repair; increasing the synthesis of Collagen I (COL-I) and Collagen III (COL-III), and their stability by down-regulating the production of the metallo-proteinases 1, 3 and 9 (MMP1, MMP3 and MMP9) [10].

3.3. Extracts with Reduced Content of Potentially Toxic Compounds

Cosmetic extracts derived from plant cell cultures perfectly suit the safety requirements that the market constantly demands. Besides being free of pathogens, pollutants and agrochemical residues, which inevitably contaminate most of the plant derived extracts, plant cells grown in the laboratory rarely contain any toxic compound or potential allergen. Many plants synthesize poisonous compounds to defend themselves against the attack of pathogens and herbivorous pests. For example, some of the compounds, known as alkaloids, are potentially toxic and are produced by several plants in response to the chewing caused by the caterpillars that feed on the plant. In absence of this type of aggression, the plant tissues would accumulate very little or no alkaloids. This is the case of plant cells that are grown in the laboratory as liquid suspension cultures: the cells are not exposed to any biotic stress (stress caused by living organisms) and as consequence no chemical defense would be activated.

A clear example of how this kind of advantage can be exploited to produce a safe active ingredient for skin care is provided by the tomato (*Lycopersicon esculentum*) cell culture extract [11]. The chemical analysis performed on the tomato cell extract revealed the absence of alkaloids, such as α-tomatine and dehydrotomatine, which are often responsible for allergic reactions and generally present in high amounts in the fruit and leaf extracts [24]. It was also rich in most of the phenolic acids and flavonoids found in tomato fruits, leaves or seeds of several varieties analyzed so far [11], and it contained phytochelatins, small metal-binding molecules, responsible for the metal binding capacity and mostly accumulated by the plant in the roots. Thanks to these chemical characteristics, the extract showed several beneficial properties on skin cells, such as oxidative stress inhibition, collagen stimulation and protection, and moreover a significant protection activity against the dangerous effects of heavy metals [25], which may cause serious damages by interacting with cellular components directly.

Similarly, extracts obtained from *Coffea* plants have long been recognized for their antioxidant capacity and immunomodulation activity [26,27]. The alkaloid caffeine, present in these extracts, is largely used in cosmetics due to its anti-fat activity and high ability to penetrate the skin barrier [28]. At the same time, it was shown that the oral administration of caffeine reduces the thickness of the hypodermis, which generally favors wrinkle formation [29]. With the purpose of developing an innovative plant cell culture extract that exhibits all the beneficial properties of *Coffea* plants, but without containing caffeine, a hydrosoluble extract was produced from cell cultures of *Coffea bengalensis*, a species that contains undetectable amounts of caffeine [12]. The authors demonstrated that the *Coffea bengalensis* cell extract was able to act independently on the three cell types that compose the main layers of the skin: (i) inducing differentiation and reducing the lipase activity in the adipocytes; (ii) inducing collagen I and III synthesis in the fibroblasts; (iii) increasing the expression of genes related to hydration in the keratinocytes, such as those of the Involucrin (INV), the Filaggrin (FLG) and the Aquaporin-3 (AQP3) [12].

3.4. Extracts Enriched in Desired Metabolites

Plant cell culture technology could also be used to obtain plant extracts enriched in specific desired secondary metabolites, at concentrations that cannot be obtained by traditional methods, such as extraction by whole plant or chemical synthesis. In this case, the cells are thus used as “biofactories”,

put in the best conditions of growth or induced to overproduce certain classes of compounds. Verbascoside, for example, a phenylpropanoid glycoside known for its antioxidant, anti-inflammatory and photoprotective properties, was produced in plant cell cultures of lilac (*Syringa vulgaris*) and butterfly bush (*Buddleja davidii*) [13,14]. The presence of high concentrations of verbascoside in the extracts of these cells allowed its further modification into a semi-synthetic derivative Verbascoside Penta Propionate (VPP), having a more hydrophilic profile and higher antioxidant capacity [14].

Another example is provided by resveratrol, a phenolic compound, member of the stilbene family, extensively studied for its large spectrum of biological activities in human health as a cardioprotective, antitumor, neuroprotective and antioxidant agent, thus widely used in cosmetics [30]. Until a few years ago, grapevine plants (*Vitis vinifera*) were still the most used source of resveratrol and its derivatives [15]. Although *trans*-resveratrol extraction from grape plants was described as promising, its extraction and purification on an industrial scale were time consuming and expensive, and thus microorganisms and plant cell cultures were alternatively used as more sustainable approaches. As microorganisms, such as bacteria and yeast, were not capable of producing resveratrol, it was necessary to introduce the genes responsible for resveratrol biosynthesis in the cells by genetic engineering, in order to have detectable levels of this compound [30]. On the contrary, *Vitis vinifera* plant cells produced *trans*-resveratrol constitutively, which made this type of system more efficient and advantageous, because no genetic modification was needed and various elicitors of the resveratrol production could be employed for increasing the yield. Various plant cell cultures have been used for the production of *trans*-resveratrol, but the most commonly used were those originating from grapevine, where *trans*-resveratrol itself was produced in larger amounts than other types of cells (5 g/L) and has been carried out using elicitation with cyclodextrins. The comparison of the productivity of recombinant microorganisms and plant cells clearly indicate that *Vitis* suspension cultures represented the most valuable system for resveratrol production [15].

3.5. Additional Advantages and Limitations of Plant Cell Culture Extracts

Another important characteristic of the plant cell culture extracts is that they contain a wide range of active compounds, and in many cases even at higher concentrations than those present in plant extracts. Even though they are used at minimal concentrations in the cosmetic preparations, the plant cell extracts can act in a synergistic manner in order to enhance a specific activity. One example is given by the mixture of liposoluble fraction of the Aglianico wine marc (LME) and liposoluble cell culture extract of *Vitis vinifera* (LCE) [16]. The grape marc represents an industrial waste product, but it is still very rich in nutrients and active molecules. In a recent study it was shown that the combination of the LME and LCE, mixed in a 10:1 ratio, gave stronger effects than either extract on its own, increasing the detoxifying capacity of the cells by up regulating proteasome activity, boosting the hydrating potential and stimulating new collagen production [16].

Plants with high nutritional values, used as food for centuries, certainly represent a rich source of compounds having a wide range of efficacy and many benefits for the skin as well. Among the legumes, *Dolichos biflorus* is one of the major crop plants worldwide and, thanks to its high nutritional value, it has been widely used as a dietary source. It was shown that the suspension cell cultures obtained from *Dolichos biflorus* plants were a rich source of active compounds, capable of protecting

the skin cells against photo-aging, both by preventing the inflammatory process and extracellular matrix degradation at the tissue level, and by stimulating the natural mechanisms of defense against UV radiation in the single cells [17].

Although undifferentiated plant cell cultures certainly represent a valuable source of extracts with proven cosmetic efficacy, they may have some limitations related to their fast growth rate and undifferentiated state, and not being the most suitable system for the production of certain classes of specific secondary metabolites. As described earlier, plant cell cultures are obtained through mitotic reactivation of differentiated cells in a given organ, generating a multicellular mixture of proliferating cells. Sometimes, depending on the plant species, such cellular assortments exhibit poor growth properties with inconsistent yields of natural products due to deleterious genetic and epigenetic changes that occur during this process [31]. To bypass this problem, innately undifferentiated cambial meristematic cell (CMC) cultures of Japanese yew (*Taxus cuspidata*) were established and their growth properties on a large scale were investigated in comparison with that of the embryo-derived cell cultures. It was proven that CMC exhibited stem cell-like properties and had a significantly higher survival rate. Also, the level of paclitaxel, a key anticancer drug produced by *Taxus cuspidata* and whose role in cosmetics has been explored [18], was 102 mg/kg of fresh cell weight, consistently higher than that generated in the *Taxus* cell suspension cultures, which was 39 mg/kg, suggesting that the CMC cultures could be a more suitable system than the undifferentiated cell suspension cultures for the production of certain specific metabolites [2].

4. Summary and Perspectives

Plant cell cultures represent a more versatile and powerful system than whole plants to obtain different types of extracts with multiple specific activities for skin care. Extracts obtained from plant cell cultures, grown under controlled laboratory conditions and in the absence of contaminants and pollutants, are completely standardized, which guarantees a high quality of sustainable product anytime. Also extracts from rare or endangered plant species can be easily produced by the use of plant cell cultures, without environmental impact and in agreement with the bio-sustainability issues that the market requires.

Thanks to these unique proprieties, the use of plant cell culture technology in skin care and functional make-up is growing rapidly. According to the last consumer and research reports from RNCOS Business Consultant Services [32], plant “stem” cell technology is expected to push forward the global cosmetic market, since it provides scientific innovation and new opportunities for product development.

Plant cell cultures certainly represent an interesting source of active ingredients for the cosmetic market, although in terms of costs they still remain expensive due to the research and biotechnological processes needed to generate and maintain them. To overcome this limitation, alternative biotechnological tools and techniques will soon become available and applied to cosmetic research in order to provide more affordable and sustainable products.

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Author Contributions

All the authors contributed to the writing and editing of the article.

Conflicts of Interest

The authors declare no conflict of interest.

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