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НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ
ФАКУЛЬТЕТ ЕКОЛОГІЧНОЇ БЕЗПЕКИ, ІНЖЕНЕРІЇ ТА ТЕХНОЛОГІЙ
КАФЕДРА БІОТЕХНОЛОГІЇ

ДОПУСТИТИ ДО ЗАХИСТУ
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ДИПЛОМНА РОБОТА

(ПОЯСНЮВАЛЬНА ЗАПИСКА)

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Тема: «Біообробка культури рослинних клітин як нова технологія виробництва косметичних препаратів»

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ALLOWED TO DEFENCE
Head of the Department
_____M.M. Baranovsky
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BACHELOR THESIS

(EXPLANATORY NOTE)

OF GRADUATING STUDENT OF EDUCATIONAL DEGREE «BACHELOR»
SPECIALTY 162 «BIOTECHNOLOGY AND BIOENGINEERING»
EDUCATIONAL PROFESSIONAL PROGRAM «PHARMACEUTICAL BIOTECHNOLOGY»

Theme: «Plant cell culture bioprocessing as emerging technology for production of cosmeceuticals»

Executor: student of group 404, FESET Verteba M.L.

Supervisor of studies: Ph.D., Associate Professor Petyukh G.P.

Standards inspector: Drazhnikova A.V.

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Faculty Environmental Safety, Engineering and Technologies

Department of Biotechnology

Speciality: 162 «Biotechnology and bioengineering»

EPP: «Pharmaceutical Biotechnology»

APPROVED

Head of the Department

_____Baranovsky M.M.

«___» _____2021

TASK

From bachelor thesis of student

Verteba Mariia Leonidivna

1. Theme of degree work: «Plant cell culture bioprocessing as emerging technology for production of cosmeceuticals» approved by the Rector «11» of May 2021 № 715/ст

2. Term of work execution: from «10» of May 2021 and «20» of June 2021.

3. Content of explanatory note: Introduction. Literature review. Materials and methods. Analysis of applied plant cell culture technique, its benefits and methods of optimization. Conclusions. References.

4. List of necessary graphic (illustrative) material: 6 fig., 1 tables.

НАЦІОНАЛЬНИЙ АВІАЦІЙНИЙ УНІВЕРСИТЕТ

Факультет екологічної безпеки, інженерії та технологій

Кафедра біотехнології

Спеціальність: 162 «Біотехнології та біоінженерія»

Освітньо–професійна програма "Фармацевтична біотехнологія"

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Завідувач кафедри

_____Барановський М.М.

«___» _____2021 р.

ЗАВДАННЯ

на виконання дипломної роботи

Вертеби Марії Леонідівни

1. Тема дипломної роботи: «Біообробка культури рослинних клітин як нова технологія виробництва косметичних препаратів», затверджена ректором від «11» травня 2021 р. № 715 / ст.

2. Термін виконання роботи: з «10» травня 2021 р. до «20» червня 2021 р.

3. Зміст пояснювальної роботи: Вступ. Літературний огляд. Матеріали і методи. Аналіз застосованої техніки культури клітин рослин, її переваги та методи оптимізації. Висновки. Списки використаних джерел.

4. Список необхідного графічного (ілюстративного) матеріалу: 6 рис., 1 таблиця.

5. Schedule

№	Task	Execution term	Signature of the head
1	Receiving and coordination of theme's task	10.05.2021 - 12.05.2021	
2	Selection of information sources	12.05.2021 - 17.05.2021	
3	Future work concept development	17.05.2021 - 20.05.2021	
4	Processing information sources	20.05.2021 - 25.05.2021	
5	Formulation of the intermediate and final foundations	25.05.2021 - 30.05.2021	
6	Structuring and composing of all the findings into a final document form	30.05.2021 - 05.06.2021	
7	Thesis defence	14.06.2021	

6. Date of task receiving: «10» of May 2020

Supervisor of degree work_____ Petyukh G.P.

Task for execution was taken over by_____Verteba M.L.

5. Календарний план-графік

№	Завдання	Термін виконання	Підпис керівника
1	Отримання і узгодження завдань роботи	10.05.2021 - 12.05.2021	
2	Підбір джерел інформації	12.05.2021 - 17.05.2021	
3	Розробка концепції майбутньої роботи	17.05.2021 - 20.05.2021	
4	Обробка інформаційних джерел	20.05.2021 - 25.05.2021	
5	Формулювання проміжних та фінальних обґрунтувань	25.05.2021 - 30.05.2021	
6	Структурування та оформлення всіх знайдень у кінцевий документ	30.05.2021 - 05.06.2021	
7	Захист дипломної роботи	14.06.2021	

6. Дата видачі завдання: «10» травня 2021 року

Керівник дипломної роботи _____Петюх Г.П.

Завдання прийняла до виконання_____Вертеба М.Л.

ABSTRACT

Explanatory note to the diploma thesis «Plant cell culture bioprocessing as emerging technology for production of cosmeceuticals», 55p., 1 table, 6 figures, 60 references.

Plant extracts are now present the most attractive source of active ingredients in cosmetics since they can provide health benefits in addition to aesthetic appearance. Plants are truly rich in endogenous bioactive compounds, known as secondary metabolites, that had found their use not only on pharmaceuticals but also in cosmetics. Phenolics, alkaloids, terpenes, flavonoids, fatty acids, polysaccharides, peptides, etc. are among remarkably potential of consisting phytochemicals.

The most part of the present active cosmetic ingredients, obtained by plant cell culture technology are sold in the form of various extracts, e.g. liposoluble or hydrosoluble, dried or plant cell wall. It is even possible to collect more than one active ingredient from the same culture by using contrasting extraction methods and solvents, and that is another advantage of the chemical nature of plant cell components.

The plant cell culture technique's basics lay mainly on the concept of plant cells' totipotency and plasticity, by their ability to generate any cells from starting tissue as well as regenerate through process of cell dedifferentiation to a non-differentiated or callus cell state and for their possible following differentiation. Hence, the most of the cosmetic ingredients obtained by plant cell culture technology are based on cultivation of de-differentiated callus or de-differentiated plant cell suspension cultures. However, the preferable method is the cultivation of plant cells in liquid medium.

Object of investigation – analysis of plant extracts production via cell culture technology using biotechnological tools.

Subject of investigation – literature.

Purpose of the work – to analyze the benefits and applicability of plant cell culture-derived extracts in cosmeceuticals.

Methods of investigation – analytical.

METHODS AND APPLICATION OF PLANT CELL CULTURE AS COSMETIC ACTIVE INGREDIENTS, COSMECEUTICAL PRODUCTION.

РЕФЕРАТ

Пояснювальна записка до дипломної роботи «Біообробка культури рослинних клітин як нова технологія виробництва косметичних препаратів», 55 с., 1 таблиця, 6 рисунків, 60 посилань.

Зараз рослинні екстракти є найпривабливішим джерелом активних інгредієнтів у косметиці, оскільки вони, крім естетичного вигляду, можуть надати користь для здоров'я. Рослини багаті на ендогенні біоактивні сполуки, відомими як вторинні метаболіти, що знайшли своє застосування не лише в галузі фармацевтики, а й в косметиці. Фенольні сполуки, алкалоїди, терпени, флавоноїди, жирні кислоти, полісахариди, пептиди та ін. Є одними з потенійно важливих хімічних складових.

Більша частина існуючих косметичних інгредієнтів, отриманих за технологією культури рослинних клітин, виготовляється у формі різних екстрактів, наприклад ліпорозчинних або гідророзчинних, висушених або екстракт клітинної стінки рослин. Можна навіть отримати більше однієї діючої речовини з однієї культури за допомогою різних методів екстракції та розчинників, і це ще одна перевага хімічної природи рослинних клітинних компонентів.

Основи методів культури рослинних клітин полягають головним чином у тотипотентності та пластичності рослинних клітин завдяки їх здатності генерувати будь-які клітини з вихідної тканини, а також регенерувати в процесі дедиференціації клітин до недиференційованого або калюсного стану клітин і їх можливої подальшої диференціація. Отже, більшість косметичних інгредієнтів, отриманих за технологією культури рослинних клітин, засновані на вирощуванні дедиференційованих каллусів або суспензії культур дедиференційованих рослинних клітин. Однак кращим методом є вирощування рослинних клітин у рідкому середовищі.

Об'єкт дослідження - – аналіз виробництва рослинних екстрактів через технологію культури клітин за допомогою біотехнологічних інструментів.

Предмет дослідження - література.

Мета роботи - проаналізувати переваги та придатність екстрактів отриманих культурою клітин рослин у космецевтичних препаратах.

Методи дослідження - аналітичні.

МЕТОДИ ТА ЗАСТОСУВАННЯ РОСЛИННИХ КЛІТИННИХ КУЛЬТУР ЯК КОСМЕТИЧНИХ АКТИВНИХ ІНГРЕДІЄНТІВ, КОСМЕТИЧНЕ ВИРОБНИЦТВО.

CONTENT

LIST OF SYMBOLS, ABBREVIATIONS, TERMS

MS – Murashige and Skoog medium

LS – Linsmaier and Skoog medium

B5 – Gamborg medium.

SH – Schenk and Hildebrandt medium

NN – Nitsch and Nitsch medium

C – carbon

H – hydrogen

O – oxygen

N – nitrogen

P – phosphorous

K – potassium

Ca – calcium

Mg – magnesium

S – sulfur

Fe – iron

Mn – manganese

Zn – zinc

B – boron

Co – cobalt

Mo – molybdenum

mM/L – millimoles per liter

mg/L – milligram per liter

PGR – plant growth regulators

Rpm – rotations per minuite

1-D motion – one dimensional motion

ca. – circa

PFP - p-fluorophenylalanine

UV – ultra violet

INV – Involucrin

FLG – Filaggerin

AQP3 – Aquaporin-3

INTRODUCTION

Actuality. 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.

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, natural, contaminant-free and bio-sustainable products, whose production can be easily extended to an industrial scale.

Object of investigation – synthesis of active compounds for further use in cosmetic formulations.

Subject of investigation – plant cell culture for production of active compounds and their use in cosmetic formulations.

Purpose of the work - to analyze the perspective of using of plant cell culture derived cosmetic ingredients as source of cosmetics' active components.

Tasks for execution of the bachelor thesis:

– To describe the plant cell culture technique for bioactive ingredients production with further use in cosmeceuticals.

– To present and analyze plant cell culture derived active substances for application in cosmetic formulations.

– To analyze the benefits of plant cell culture-derived active ingredients and methods of their production optimization.

Methods of investigation – analytical.

ВСТУП

Актуальність. Рослини - це унікальні організми, здатні створювати власну їжу завдяки фотосинтезу та забезпечувати киснем атмосферу. Вони також є важливими джерелами їжі та терапевтичних сполук для всіх інших живих організмів. Рослини відрізняються високою синтетичною універсальністю: спектр хімічних структур, синтезованих рослинами, ширший, ніж у будь-якої іншої групи організмів, що робить рослини найбільшим джерелом природних засобів у фармацевтичній, харчовій та косметичній галузях.

Через великий попит на природні сполуки постійно зростаючим світовим населенням, комерційно цікаві рослини дедалі більше культивуються в промислових масштабах; але в багатьох випадках методи посіву та методи видобутку не були оптимізовані, і, як наслідок, весь процес виявився економічно нежиттєздатним. Культури рослинних клітин, безумовно, є вагомою альтернативою для виробництва косметичних активних інгредієнтів, оскільки вони завжди забезпечують стандартизовані, природні, не забруднюючі та біостійкі продукти, виробництво яких можна легко розширити до промислових масштабів.

Об'єкт дослідження - синтез активних речовин для подальшого застосування в косметичних препаратах.

Предмет дослідження - культура рослинних клітин для виробництва активних речовин та їх застосування у косметичних препаратах.

Мета роботи - проаналізувати перспективу використання косметичних інгредієнтів, отриманих із культури рослинних клітин, як джерела активних компонентів косметики.

Завдання для виконання дипломної роботи:

– Описати техніку культури рослинних клітин для виробництва біоактивних інгредієнтів з подальшим використанням у косметиці.

– Представити та проаналізувати нові активні речовини, отримані з рослинних клітин, для застосування в косметичних продуктах.

– Проаналізувати переваги активних інгрідієнтів, отриманих культурою рослинних клітин та методі оптимізації їх виготовлення.

Методи дослідження - аналітичні.

CHAPTER 1
LITERATURE REVIEW

1.1. Plant extracts as active ingredients in cosmeceuticals

Plants are a prosperous source of endless metabolites that have an enormous potential in cosmetic products' application. Since bygone days, the mankind has been using various plants and their extracts in order to create cosmetics with the intent of establishing a state of eternal youth [1].

Plant extracts are currently turning into the most attractive source of active ingredients in cosmetics due to the ever-growing demand for natural compounds as well as they can provide health benefits in addition to aesthetic appearance. The advancements in suchlike products, called "cosmeceuticals", reflect the latest trend in the cosmetics and personal care industry these days. Plants are truly rich in endogenous bioactive metabolites that had found their use for cosmetic and pharmaceutical purposes (Fig. 1.1). Phenolics, terpenes, flavonoids, stilbenes, steroids, saponins, terpenes, fatty acids, polysaccharides, sugars, peptides, etc. are among remarkably huge number of these phytochemicals. They can be extracted with pertinent solvents for further use as active ingredients in cosmetic formulations [2]. Due to this, enormous numbers of plant sources have been examined by the cosmetics industry in look for novel active ingredients that could merge particular pharmacological properties. Antioxidant, antimicrobial, antiviral, anti-inflammatory, anti-allergy, as well as decent moisturizing, anti-ageing, and ultra-violet protective properties are among them and the most gainful [3].

The impelling course behind the swift progress in the development of plant cell culture-derived active ingredients for the demand of the cosmetics industry consist of the vast survey for innovations and elaboration of new products with aliquot concrete properties. Multiple biological activities make plant cell culture extracts are highly preferable because of the fact of an uncommon combination of secondary and primary metabolites that occur naturally in plant cells. Besides, the final cosmetic products commonly contain active

ingredients in very low concentrations. This feature allows the manufacturing of plant cell extracts in small amounts at tolerable prices that cover production costs [4-6].

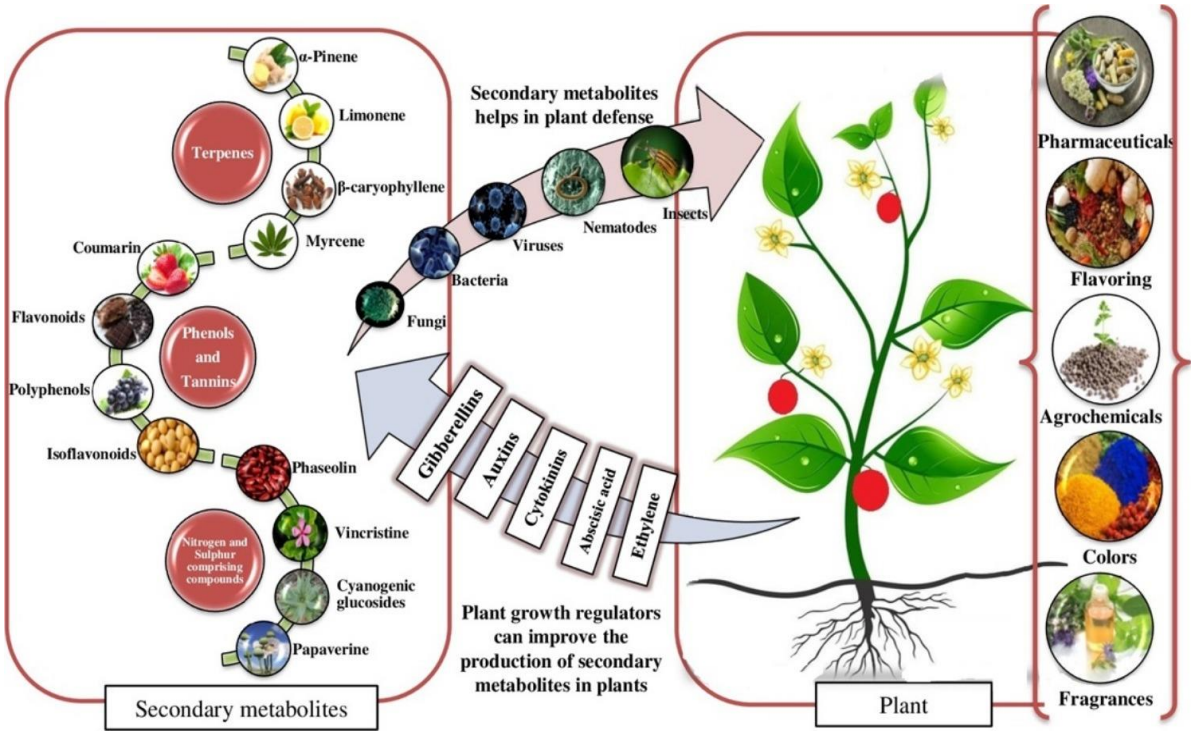

Fig. 1.1. Endogenous bioactive metabolites present in plants [25]

The most part of the vacant active cosmetic ingredients, obtained by plant cell culture technology are sold in the form of various extracts. In comparison to the plant – derived extracts, plant cell culture-obtained extracts can be freely standardized, fully satisfying required safety demands. Plant cell extracts are manufactured under controlled conditions and thus do not contain pathogens, toxic compounds, allergens and pollutants [7-9]. The type of solvent used divides plant cell extracts into:

- liposoluble (extracted with oils),
- hydrosoluble (extracted with glycerin) extracts,
- dried extracts (conditioned with maltodextrin),
- plant cell walls extracts (peptides and sugars enriched),
- nanoemulsions or suspension extracts [10,11].

Moreover, it is even achievable to collect more than one active ingredient from the same culture by using contrasting extraction methods and solvents, and that is another advantage of the plant cell components' chemical nature.

1.2. Plant cell culture basics

The technique's basics lay mainly on the concept of plant cells' totipotency that appeal to a generation of any cells from starting tissue [9]. *In vitro*, plant cells have incredibly immense level of plasticity: tissues, organs, and even entire plants can be regenerated through cell dedifferentiation process to a stem cell state, and then to differentiation that follows. Plant cell suspension cultures, also called plant 'stem' cell cultures, could be a particularly valuable source of commercially valuable secondary metabolites, which are normally synthesized in low amounts in differentiated tissues and can be unequally distributed in the organs, such as leaves, roots, flowers, or fruits [14-16].

However, plant cell suspension cultures might not be the best fitting system for producing certain classes of active ingredients. In this case, cell cultures of differentiated plant cells could be possibly seen as a source. For instance, cell cultures of roots, somatic embryos, and seedlings are highly specialized and therefore are able to synthesize compounds that are not produced in undifferentiated cells, thus can be used to address more defined and selective functions in cosmetics. Thence, most part of the existing outlines for the production of plant cell cultures are developed not on the cultivation of real plant stem cells, but on the basis of the use of dedifferentiated plant cells [17, 19]. This fact made it possible to generate almost unlimited numbers of plant cell lines with unique phytochemical profiles and growth characteristics even from the same plant, used for their initiation. Therefore, expressions such as "root stem cells", "leaf stem cells", "meristem stem cells", "rhizome stem cells", "flower stem cells", "fruit stem cells", etc. can very often be considered as names of active cosmetic ingredients, but in reality each and every of them means dedifferentiated plant cell cultures [18, 20].

Yet another essential fact that concerns the plant cells used for cosmetics, states that the “plant stem cells” term is identically used for active ingredients, synthesized by either cell suspensions, callus cultures or hairy roots [21]. It should be said that callus cultures are plant cells that are cultured on solid media, whilst the cultures of cell suspension are either single plant cells or small packed cells that are cultured when immersed into in a liquid medium. Either callus or cell suspension cultures may contain dedifferentiated or true stem cells [22].

Conversely, cultures of organs, procured by genetic alterations of plant cells are hairy roots [23, 34].

1.3. Plant cell culture media

Plant cell culture media must contain the following constituents for the further growth and development of cells or tissues into the undifferentiated mass of the callus (Fig. 1.2):

- water
- macro- and micronutrients,
- vitamins,
- hormones,
- growth regulators,
- amino acids or nitrogen supplements,
- source(s) of carbon
- undefined organic supplements, and
- solidifying agents.

Ingress to the atmospheric oxygen for gaseous exchange and the desirable pH must be also ensured.

It ought to be noted that the optimal quantity of every nutrient for reaching crest growth rates depends on the species. Each plant strain requires its own conditions. Certain plant strains readily grow on simple media while others necessarily require addition of vitamins, growth factors, and specific selective substances.

Basic media that are frequently applied include Murashige and Skoog (MS) medium [1], Linsmaier and Skoog (LS) medium [3], Gamborg (B5) medium [4], Schenk and Hildebrandt medium (SH), and Nitsch and Nitsch (NN) medium [25].

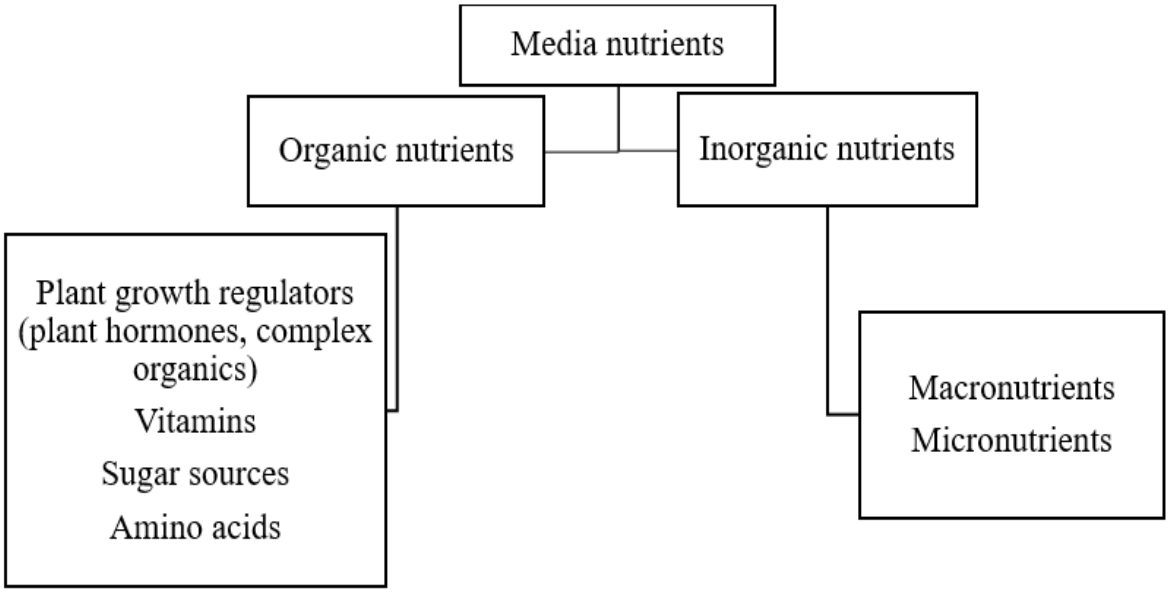

Fig. 1.2. Outline of media main components [60]

Macro- and micronutrients. *In vitro* cultivation of plants also demands a mix of macro- and micronutrients as much as for *in vivo* growth. Their level of presence is given in the image of millimolar number. The elements having a concentration that is more than 0.5 mM/L are named as macro-elements and elements having a concentration under 0.5 mM/L are named as microelements [26].

Vital elements in plant cell or tissue culture medium consists of, apart from C, H and O, macroelements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) needed for their growth and metabolism. Culture medium must contain leastways 25-60 mM of inorganic N for reasonable growth plant cell. K is essential for almost all plant species. Majority of medium contains K in the form of salts of nitrate chloride having the range of concentrations between 20 and 30 mM. Diapason between 1-3 mM is the most favorable concentration of P, Mg, S and Ca [26].

Vital micronutrients for growth of plant cells and tissues consist of iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo). Iron is generally

the most crucial among these micronutrients, and is added in the form citrate or tartarate salts. Cobalt (Co) and iodine (I) may be appended to some media, but not necessarily. Sodium (Na) and chlorine (Cl) are also added to certain media, despite of they are not that substantial for growth. Copper and cobalt are appended at concentrations of 0.1µM, Fe and Mo at 1µM, I at 5µM, Zn at 5-30 µM, Mn at 20-90 µM and B at 25-100 µM [26-27].

Carbon and energy sources. In plant cell culture medium, sucrose is often used as source of carbon contributing 2-5%, but some other carbohydrates can be used as well. These may be galactose, maltose, lactose, and starch. However, they are less efficient comparing with sucrose or glucose [27-28].

Addition of banana extracts, sugar cane molasses, and coconut water to basic medium may be a nice disjunctive in order to reduce primary costs. These substrates also contain vitamins and inorganic ions that are demanded for growth [28].

Vitamins. Vitamins are organic agents that are essential for metabolism of cells in the form of cofactors or enzymes' constituents. Nicotinic acid (B_3), thiamine (B_1), pantothenic acid (B_5), and pyridoxine (B_6) are generally used vitamins of which thiamine (0.1 to 5 mg/L) is always appended to the media because it takes part in the metabolism of carbohydrates. Nicotinic acid is appended at a concentration of 0.1-5 mg/L and pyridoxine at 0.1-10 mg/L. Some others like riboflavin, ascorbic acid, pantothenic acid, biotin, folic acid, tocopherol (vitamin E), p-amino-benzoic acid may be added but their amount presence are not crucial [26-27].

Amino acids. Their mixtures such as casein hydrolysate, L-glutamine, L-asparagine and adenine are often used as founts of organic nitrogen in culture media. Casein hydrolysate is usually added at concentrations of 0.25g/L. Glycine at 2 mg/L, glutamine up to 8 mM, asparagine at 100mg/L, L-arginine and cysteine at 10 mg/L and L-tyrosine at 100mg/L are amino acids added for amelioration of growth of cell in culture medium [27].

Complex organics. Complex organics can be as well named as undefined or natural supplements. They are a bunch of undefined adjunction like yeast extract, coconut water, casein hydrolysate, orange or tomato juice, etc. Activated charcoal can sometimes be added as well whether is needed or not. Adjunction of natural origin has advantageous impact on cultures of plant cells and tissues *in vitro*. Organic adjunction is necessary for expedient

plant expansion and regeneration. These complex organics are a valid fount of certain vitamins, hormones, amino acids, fatty acids, carbohydrates, and some other plant growth regulators. Their optimal quantity depends on the plant and species. Addition of compex organics isn't integral nor crucial but is frequently advantageous [26, 27-28].

Solidifying agents. These are applied in order to prepare semisolid culture medium to permit explants to be located on culture medium to grant bountiful aeration. Agar, agarose and gellan gum are the most widely used.

Agar, that is a polysaccharide acquired from seaweeds, is of all-purpose usage as a gelling mean for preparing semi-solid and solid medium [29].

Plant growth regulators. Plant growth regulators enhance cell partition and thus control the growth and differentiation of explants' roots and shoots in semisolid and liquid medium cultures [29-30]. Addition of PGRs in different cultures results in the expression of various genes (factors of transcription) that then controls the various phases of growth (regeneration of shoots, roots, or calli from root explants) in plants. Major kinds of plant growth regulator applied are:

– Auxins - indole-3- acetic acid (IAA), indole-3- butricacide (IBA), 2,4-dichlorophenoxy-acetic acid (2,4-D) and naphthalene- acetic acid (NAA); promotion of callus production and growth of cells, commencement of roots and shoots, promotion of growth from shoot apices and shoot stem cultures,

– Cytokinins - BAP (6-benzyloaminopurine), 2iP (6-dimethylaminopurine), kinetin (N-2-furanylmethyl-1H-purine-6-amine), Zeatin (6-4-hydroxy-3-methyl-trans-2-butenylaminopurine) and TDZ (thiazuron-N- phenyl -N-1,2,3 thiadiazol-5ylurea); these ones promote division of cells, provoke shoot formation and axillary shoot propagation,

– Gibberellins – consist of more than twenty compounds, of which GA3 is the most frequently used gibberellin; these compounds enhance growth of callus [13] and help elongation of dwarf plantlets.

– Other growth regulators are appended from time to time to medium. For instance, an abscisic acid, a substance that is generally added to suppress or induce callus growth, this depends on the plant species or ethylene amount that is present. Ethylene is a

PGR of natural origin, that originates in a gasiform state and generally related to controlling the ripening of fruit in climacteric species of fruits. Its liberation in certain cultures of plant cells frequently suppresses the growth and extension of the culture. To get rid of this compound in the gasiform state, 2-chloroethane phosphoric acid in the form of powder is appended to release ethylene. It induces shoot propagation and suppresses further stages of fetus extension [18]. Even though PGRs are the costliest medium components, they carry slight impacts on the medium cost due to their small concentration requirements.

pH. Most favorable pH for culture medium is 5.6–5.8 before the process of sterilization. The number of pH less than 4.5 or more than 7.0 noticeably suppresses growth and extension of cells and tissues *in vitro*. In case the pH incidence considerably during cultivation (pH under 5.0 doesn't let agar to turn into gel and thus the medium turns into liquid), then a brand-new medium ought to be made, whilst a pH more than 6.0 results in hard medium (intervenes with nutrients uptake). Also it is well-knows that the media pH impacts the agar gelling capability, absorption of ingredients, various salts solubility, and occurrence of chemical reactions (particularly those that ate catalyzed with the help of enzymes) in the media [23].

1.4. Principles of plant cell culture bioprocessing for production of active cosmetic ingredients

Neoteric techniques for giving rise of metabolites from plant cell cultures are presented in Figure 1.3. Even though some plants (especially monocotyledons) are more intractable than others, now it is possible to induce callus cultures from virtually any plant species. Plant tissue culturing allows the propagation of undifferentiated plant cells either to revive a whole plant or to produce single cells in culture for further production of secondary metabolites [29].

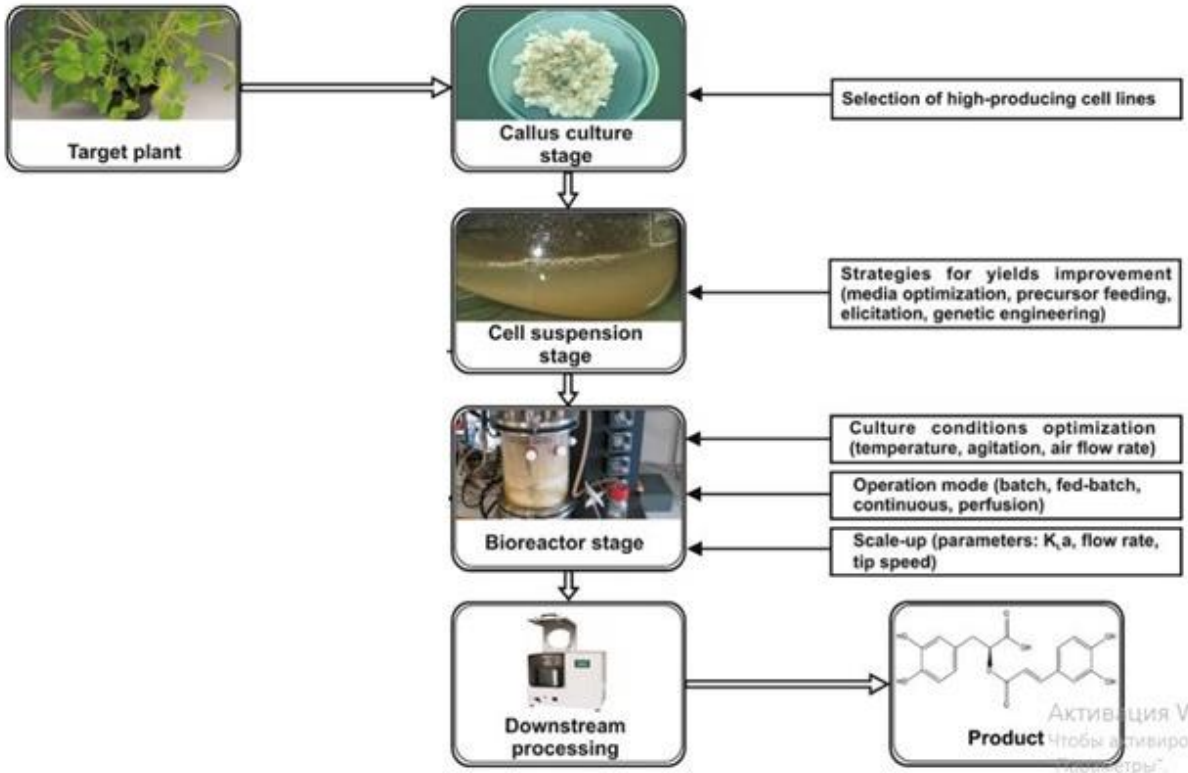

Fig. 1.3. State-of-the-art technological platform for plant cell culture [22]

The plant tissue material being initiated as an origin for plant cell culture denominates as an explant. Almost all plant tissues can be used as explants. After wounding, the volume of explant's surface cells starts to increase. Afterwards these cells divide, dedifferentiate and a mass of cells called callus is formed. Oftentimes, a location of a formed callus is the cut end of the stem or root that proceeds in a result of wounding. Approximately it is an unnorganized tissue which consists of both undifferentiated and differentiated (beginning matter) cells. Hence, the word 'callus' means cells containing differentiation of varying extent. Therefore, callus cultures are actually cell lumps elevated because of unorganized propagation of cells from the certain regions of organs of plants [30].

With this method, plant cells multiply in an appropriate environment to form clusters of undifferentiated cells called callus. When callus has differentiated cells that are present in isolated explants, then dedifferentiation has to occur (ie, the cells go through changes in order to become meristematic) right prior to cell division that takes place. If explants contain only meristematic tissue in an isolated state, afterwards cell division occurs without dedifferentiation, if only meristematic tissue is contained in an insulated condition. While

the process of dedifferentiation takes place, matured cells are temporarily capable to go back from adult to the adolescent state. The readolescented cells have a bigger rise and possibility to divide. By means of specific occasions these cells can regenerate these organs. Consequently, an eminent step to take into consideration at *in vitro* proliferation of plants is this dedifferentiation. Annex of appropriate inducing hormones often accompanies the initiation of callus, as well as the augmentation-supporting medium and sterile environment. Purvey of external growth adjustors that are present in the nutrient medium impacts the forming of callus from the initial explants. It is possible to divide the purvey of hormones for callus growth into three passages (auxin and cytokinin separately, or auxin and cytokinin combined) [21-22].

The callus can be sustained *in vitro* technically for indefinite period with the help of the right growth medium; but, prosperous callus rise depends on the plant species and ensured environment. If the callus is subcultured on the fresh medium, then this dedifferentiated cell mass is provided with unlimited preservation time.

If cells of callus are cultured in liquid media, they shape a rapid growing suspension culture of single cells or tiny lumps of cells [21].

Suspension culture is the manipulating of insulated cells in liquid medium. Any explant is suitable for insulation of cells from induced callus. This is the most general method to introduce suspension cultures from callus that already grows within the culture. Widely callus cultures are subdivided to two diverse classes: friable and compact [29-30].

Densely aggregated cells are present in compact callus while loosely associated cells are in friable callus. Suspension cultures are generated by the means of the kind of callus. These callus' kinds suggest the inoculums in order to shape cell suspension cultures. To initiate cell suspensions, quite broad inoculums must be applied so as the liberated concentration rapidly enlarges. These kinds of cells depict an inferior level of organization than tissue or callus culture and are usually called cell cultures. This liquid medium is usually alike in content to the one of callus growth. But, regulations have to take place in the percentage of hormones and inorganic salts contents. Requirements of agitation are also considered. This is made because of three goals: the cell aggregates breakage; a uniform

distribution of different cells maintenance; and cell lumps in the media provide gaseous exchange for the cells to support cell respiration in the liquid media [30].

During the procedure of cell division, the inoculums split and pour new cell lumps, that break down again to provide separate cells and any other tiny groups. Because of the natural propensity of plant cells to stick together, it is not always eventual to proliferate a suspension of single cells only. Various kinds of suspension cultures are presented in Fig. 1.4.

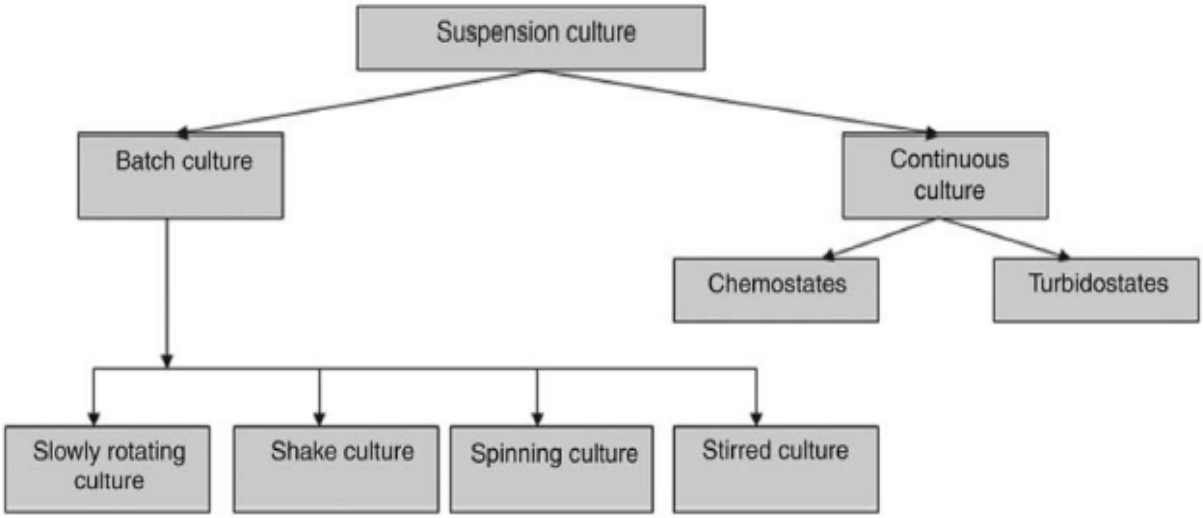

Fig. 1.4. Types of suspension culture [60]

Batch culture is a closed system of culture that has a confined nutrients' supply. In this type of culture, cells rise in a confined volume of liquid media and are generally held in conical flasks on orbital shakers at an 80–120 rpm velocity. A bunch of various kinds of batch cultures exist – shake, centrifuge, slow rotating, and shaken culture [30].

Cultures requiring a constant purveyance of a cell suspension or product in their media are called continuous cultures. This type of structure is sustained in a stable state for prolonged durations by means of draining the wasted liquid media out and appending clean media in order to steady the physiologic condition of cells under growth. These media were made such wise that one of the components is confined, thus at expository growth, when the nutrient is exhausted, this growth brings to stop. Nevertheless, afore the exhaustion of nutrients, pure nutrient-confined medium is supplied. Because of the ongoing flow of nutrients and the stable condition of the cells, nutrient exhaustion is absent. Development of

bioreactors of various sizes and configuration surely took place for continuous cultures with their dependence on the plant cells' diversification [16-17]. Two kinds of continuous cultures exist: (a) *closed continuous culture* – by this manner, cells are detached from drained media and returned to the suspension culture and (b) *open continuous culture* – by this manner, harvesting of identical volume of suspension culture goes along with supplementation of the medium.

Cultures of plant cells cultivated in a liquid medium are then up-scaled to abundant bulks and applied for the manufacture of profit-oriented components for industrial practice, cosmetic formulations indeed [32].

1.5. Plant cell culture propagation and extract manufacture

The obtained callus is constantly shifted to an expedient nutrient medium that must include all the substrates essential for metabolism of cells. The culture takes place in the dark and hence the culture forfeits its capability to photosynthesize and thus starts to behave as a heterotrophic being. During this step, the culture is provided with a substance that acts as a fount of organic carbon and energy (almost always a saccharose) and plant grows regulators (auxin and cytokine), vitamins and as well as micro- and macroelements mentioned above.

Due to the nutrient composition variations, cell lines with various features can be obtained, and later on the cell line having the best biochemical and metabolic characteristics is selected. Reiterated transfers are being carried out until the moment of acquiring the cell line with the most undeviating and unified features. It is adequate to indicate that the acquired cell line is not altered genetically, and its screening is based fully on morphological, biochemical features and on the callus tissue growth capability. Fermentation is controlled by a pattern metering of sugar and pH levels, cell vitality and bulk of the generated biomass [32-33].

The significant component of the manufacture process shifted to the industrial scale is adjustment of the chosen cell lines for growth in a liquid nutrient which makes a significant increase of a biomass bulk possible. The suspension cultures demand piecemeal

adjustment for growth inside a cone flask (volume ca. 200 mL) and after to grow inside bioreactors which guarantee bigger capacity (volume up to 100 L and even up to 300 L). Scaling is the name of this process [19-20].

The choice of the bioreactor type that is the most corresponding for an exclusive bioprocess is a very complicated mission since the most favorable type of bioreactor ought to be well-implemented as much as extensible and ought to maintain the growth of the generating cell line and the synthesis of the desired bioactive compound(s) whilst holding the bioreactor imprint as low as possible.

This implies that mixing gas provision and plant cell culture diffusion must be bountiful, whilst mass transfer restraints and stockpiling of adverse by-products ought to be shunned [5]. Superfluously elevated shear forces inside the bioreactor, that come from high particular power input by means of agitation and/or aeration, need to be expelled. Uniform and abundant luminosity and heat diffusion for photoauto- and photomixotroph plant cell cultures in the bioreactor are demanded as well.

Plant cell suspension lines, notably those that grow leisurely and behave as Newtonian fluids, are trouble-free to disseminate in bioreactors. Enabling of significant power capacity and oxygen provision must be emphasized in case of plant cell cultures with non-Newtonian fluid flow demeanor (generally rapid growing plant cell suspension cultures). And they should be propagated sans detriment caused by hydro mechanical stress [7].

Concerning all the above, a wide variety of bioreactor designs has been applied for cultivation of plant cell cultures. The conventional bioreactor arrangements (e.g., stirred tank reactors, airlift reactors, and bubble column reactors) applied for *in vitro* cultivation of plant cells were replicated from those that had been designed and had found their usage in microbial biotechnology, in addition to wave-mixed bioreactor with 1-D motion [7]. The operating tenets of these four bioreactor types are depicted in Fig. 1.5.

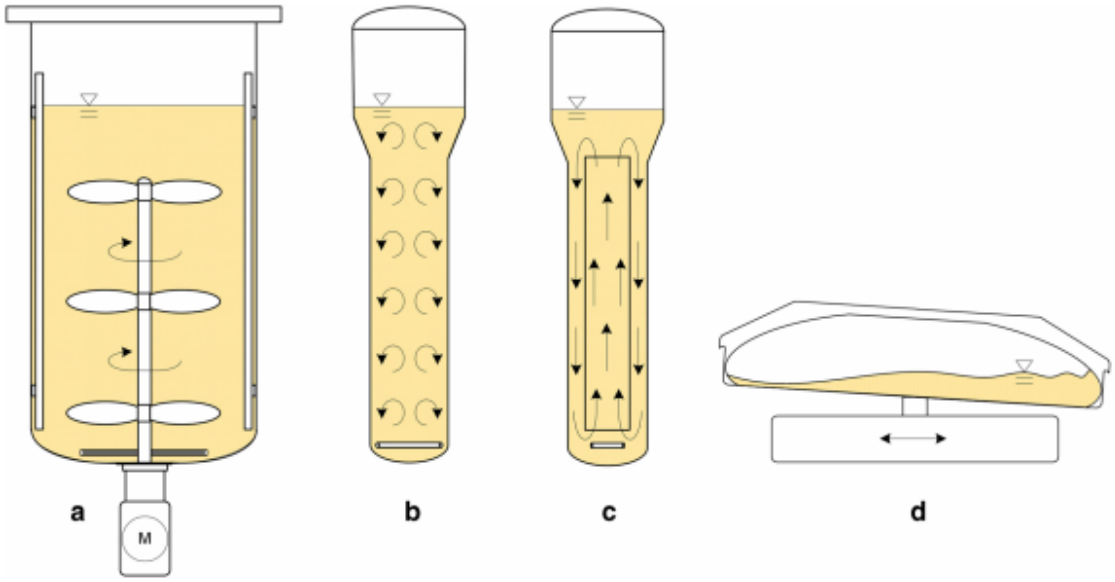

Fig. 1.5. Schematic diagrams of instrumented bioreactors preferred in commercial production processes with plant cell cultures which generate products for the cosmetics industry; *a* - stirred bioreactor, *b* - bubble column bioreactor, *c* - airlift bioreactor, *d* - wave-mixed bioreactor with 1-D motion [7]

If centering on the cubic meter scale, predominance goes to stainless steel stirred bioreactors (Fig. 5a). They pertain to mechanically driven bioreactors and are generally considered to be system of selection for plant cell suspension cultures. In opposite to their stirred complements, reusable bubble column (Fig. 5b) and airlift bioreactors (Fig. 5c) do not have moving sections. They are air-driven and are applied for mass distribution of more shear susceptible plant tissue cultures (cultures of hairy roots and somatic embryos) up to scale of cubic meters. Alterations of bioreactor, such as an augmentation in diameter of the bioreactor top partition to promote the foam decrease, have been brought into existence. Bubble column, airlift bioreactors and suchlike with a balloon-like top partition are frequently related to as “balloon-type bioreactors” [7].

Cultures executed inside bioreactors must be provided with invariable temperature and they must be mingled with an eye to guarantee suitable gas exchange degree that is needed for metabolism of cells. The growing biomass is checked by metering of sugar and pH levels, dispatch, optical density, viability of cells and secondary metabolites’ content of such as e.g., ursolic acid [41].

Plant cell culture extract manufacture. The following procedure of extract manufacture after cultivation in a bioreactor extremely depends on the chemical nature(s) of the bioactive substance(s) that will be present in the future extract of plant cell culture. Besides, whether the extract is a liquid or a powder needs to be taken into account [41]. A distinction is made between hydrosoluble (e.g., amino acids, glucides, flavonoids, anthocyanins, phenolic acids) and liposoluble (e.g., vitamins, tocopherols, fatty acids) compounds and those that were derived from plant cell walls (peptides and sugars mingle). Regardless of the kind of extract, the very first stride is perennially to gather the culture broth that contains the target compound(s) that are intracellular. The ensuing steps are producer peculiar but commonly include gathering, integrating and rupture of the cell mass, extraction with the help of solvents or proteolytic enzymes or/and methods of chromatography, followed by washing steps [42].

Therewith, if the extract is in a form of powder, a drying operation with freeze, spray, or vacuum dryers is necessary. Some manufacturing processes for plant cell culture extracts also exist that are unlike the supra, in this case, their details are located in the patent documents of every and each manufacturer [43].

It is substantial that plant cell culture extracts that are used for the cosmetics industry are not of pernicious perturb when it is the time of their ultimate application. Some substitutions take place in the means of convenient and synthetic phytohormones in the culture media, for instance, 2.4-dichlorophenoxyacetic acid, 6- bezylaminopurine, N6-furfuryladenine are substituted with natural phytohormones, like indole-3-acetic acid, zeatin as well as the use of phytohormone elicitors (jasmonic acid, methyl jasmonate, salicylic acid) and/or light onwards enhances the safeness of plant cell culture-derived products for application the cosmetics industries [45].

Even though the material from plant cell cultures is quite uncomplicated, in comparison to an entire plant, it is still a sophisticated array. The pecto-cellulosic tissue and membranes that surround organelles inside cells constitute considerable hurdles for secondary metabolites to traverse before their extrication into the liquid media. Hence, as ever a species was selected and a strain chosen to generate the goal metabolite(s) at elevated levels, or leastways comparatively elevated ones, the process of extrication must be

attentively selected to enhance initial recuperation. When in solution, the blend of secondary metabolites that has to be refined, because just one or a couple of molecules are generally of concernment [44]. Most attention must be paid in initial stages of the process of purgation, when deprivations of the metabolite(s) are essential. The process's scope (number of pure metabolite to be reckoned) is a crucial qualifier of corresponding approaches to employ and the demanded means. The whole process ought to be as rudimentary as possible, especially when scale-up follows.

The relevant procedures and setup for extrication of targeted metabolites depend on their physico-chemical properties and their intended use, e. g., *Echinacea* alkamides can be extracted with the help of polar organic solvents (methanol or ethanol, which contemporaneously deactivate enzymes) or a water-ethanol mingle having an elevated ethanol percentage; whilst, polysaccharides may be extracted with the help of water or buffer. Usage of solvents which can result in interplay that leads to changes in structure, like alkaloids with chloroform or electrophilic groups with methanol, must be circumvent [41, 43-45]. The initial application of the insulated metabolite has also to be taken into account because certain solvents are prohibited or severely controlled in cosmetics treatment.

Generally applied solvents are methyl or ethyl acetate, methyl tert-butyl ether, 1- or 2- butanol, and 2-methyl tetrahydrofuran. Such solvents like hexane, benzene, and chloroform must be avoided due to their toxicity, and can be replaced, to some degree, by heptane, toluene, cyclohexane, or methylene chloride [44].

Alternatively, biomass can be extracted in a dried state, obtained through heating, or freeze-drying. Dried, ground biomass can be directly extracted with organic solvents depending on the polarity of the metabolite(s) of interest [43].

1.6. Conclusion to chapter

Nowadays systems of cell cultures, particularly cell suspension alias callus culture, found their application for extensive culturing from which it is possible to extract secondary metabolites of interest with further addition to cosmetic products. A suspension culture is propagated by the shift of comparatively crumbly callus portion into the liquid media and

then is sustained under appropriate stipulations including agitation, aeration, illumination, temperature and some other physical criteria [36]. One of the pros of such technique is that it may eventually grant a constant and secure fount of natural products [38]. Another important avail concerning cell cultures lays in generation of biologically active secondary metabolites, managed under regulated environment, that does not depend on soil, water or climate background [39]. Bioreactors of various kinds have been applying for plant cells bulk farming.

For instance, cell of *Catharanthus roseus*, *Dioscorea deltoidea*, *Digitalis lanata*, *Panax notoginseng*, *Taxus wallichiana* and *Podophyllum hexandrum* have been farming in those bioreactors with the aim of manufacture of plant secondary metabolites.

CHAPTER 2
MATERIALS AND METHODS

2.1. Features of plant cell culture-derived compounds applied in cosmeceuticals

A cosmetic recipe that includes active ingredients of stern natural occurrence, is created to protect the skin from exo- or endogenous adverse factors, in addition to stabilize the lipids of dermal homeostasis resulted in dermatosis or ageing. Its peculiarity lays in a lipid content that is similar to oily secretion of the sebaceous glands of humans. Hence, the phospholipids' chemical content grants defense against antioxidants, naturally-occurring sun-in locking impact, anti-inflammatory and anti-free-radical molecules that can, for instance, avert histidine from decarboxylation to form histamine [35].

Moisturizing natural treatments frequently consist of mucilage, that contains polysaccharides, polyatomic sugars and starch derivatives that alleviate the skin dryness and grant a calming layer that protects the skin. Shielding of the skin level of hydration, and generating calming actions to skin and hair enrichment is obtained by means of seed oils usage that are rich in fatty acids and triglycerides that decrease trans epidermal aqua-deprivation [46]. The plants having anti-inflammatory characteristics usually contain a significant amount of flavonoids; those that are applied to rigid and timbre the skin contain tannins having a constricting impact; also another benefit for curing of skin infectious conditions is application of plants having numerous fungi- or bactericides.

Application of phytochemicals or extracts having biological activity that origin from a number of botanicals in cosmetic products fulfills two tasks: provision of body care and as constituents to impact biotic role of the skin, supplying with nutrients for fine skin, so it conjoins the cosmetic purpose and certain health function, like nourishing with anti-inflammatory or anti-allergy function, or accelerating production of collagen while stimulating process healing [40]. Commonly, botanical issues are an affluent fount of vitamins, antioxidants, essential oils, polysaccharides, lipoproteins, terpenoids and various

other biologically active substances [41]. Their composition defines the properties these extracts supply.

Even though various researches reporting the production of secondary metabolites using callus cultures and differentiated tissues [23, 34, 43], in most cases, undifferentiated cells are the preferred culture system, as seen in Table 2.1.

Table 2.1

Some of the most popular plant cell culture technology – derived active cosmetic ingredients, currently available on the market

Plant species	Type of cell culture and extracts	Benefits
Perilla frutescens	cell suspension extract	anti-aging, antimicrobial, soothing effect
Vitis vinifera cv. *Verdejo*	liposomal complex of cell suspension extract	antioxidant, moisturizer, hydrating effect
Calendula officinalis	cell suspension extract or emulsified cell suspension	anti-wrinkle, skin regeneration, deep hydration and moisturizer
Rhodiola rosea	callus culture extract	antioxidant
Malus domestica	liposomal complex of cell suspension extract	anti-wrinkle
Polianthes tuberosa	callus culture extract	anti-aging, anti-wrinkle, anti-circle, anti-puffines, brightening, firming, soothing, moisturizing
Rosa damascena	glycerin based leaf cells extract	anti-aging, firming
Paeonia lactiflora	powdered leaf cells	mattifying, radiance, moisturizing, soothing, relaxing

2.2. Active ingredients from plant cell suspension cultures

Plant cells, cultivated as suspension in a liquid medium, generate a huge number of flavonoids. Their part in cosmetic products is clearly consolidated. Flavonoids, like anthocyanins, flavonols, flavones, *etc.*, and their derivatives present the well-known due to their antioxidant features; they have the abilities to neutralize reactive oxygen species and can switch on the main genes that take part in protection against oxidative stress and harm corection in various kinds of skin cell [17]. Some types of non-flavonoids (phenolic acids, lignins, stilbenes) and carotenoids consist of free-radical-scavenging molecules likewise, that are efficient in averting formation of free radicals by collecting them or encouraging their further decay. Several substances suppress the commencement or spread of oxidative chain reactions, hence hindering or fixing oxidative destruction caused by cells of the body that were assisted by oxygen.

In addition, glycoproteins of plant cell wall membrane may be triggered in response to skin tissues damage or some other kinds of biotic and abiotic stresses. Scientists evolved appropriate extraction, purification techniques, as well as treatment of these glycoproteins with an eye to receive a blend of tiny peptides containing high amounts of proline, hydroxyproline, glycine and saccharides, that might be actually efficient in operating defense rebound cascades in skin cells of humans.

Protection and prevention of ultraviolet (UV) light harmful effects and damages is another important concern of botanicals application. A hydrosoluble extract of cell cultures of the bean species *Dolichos biflorus* that rich in isoflavone (that also includes genestin and daidzen, and their glucosidic derivatives) is one representative [38]. The extract was able to capture free radicals and block generation of collagenase in the dermis, essentially decreasing harmful impact on the proteins of extracellular matrix and maintaining collagen framework up to 72 hours after ultraviolet effulgence [39].

A second example plant cell culture-derived extract for cosmetic formulation derives from *Daphne odora* (winter daphne), a compact evergreen shrub possessing an ability to endure low temperatures. Its hydrosoluble extract contains some flavonoid compounds, like kaempferol and glucosidic derivatives, luteolin and daphnodorins. As well having

significant amount of lignans, especially wikstromol, pinoresinol, and lariciresinol, that possess anti-inflammatory effects [37]. This current extract is capable to adjust the enzyme of sebum regulation, 5α reductase 1 in fibroblasts, as well as to suppress the pro-inflammatory cytokines IL-1β and IL-8. In addition, this extract can hasten the cut curing ability of skin cells, by the pattern of causing the generation of actin and fibronectin [41].

It was mentioned previously that usage of various solvents and extraction techniques allows to isolate more than one biologically active compound from the same culture. Such example is granted by *Rubus ideaus* (raspberry) cell cultures [42]. Generally, species of *Rubus* have a high amount in water-soluble substances, like as amino acids, glucides, oligoelements, various phenolic substances, as much as oil-soluble compounds, like vitamins, tocopherols, and fatty acids, particularly linoleic, palmitic, and stearic acids. Whilst the hydrosoluble extract, thanks to the presence of phenolic acids, flavonoids, ellagic acid, and anthocyanins, resulted in a decent anti-inflammatory activity in the skin, the liposoluble partition, rich in long chain fatty acids and phospholipids, presented moisturizing effects. The anti-inflammatory activity caused by the hydrosoluble extract was to a great part because of a decline in the synthesis of the activated genes of Nitric Oxide Synthase 2 and the Cyclo-oxygenase 2 inside the cells of skin, stamping it as prospective biological constituent in order to avert inflammations and striving a calming effect on ultra violet harmed skin erythema [43]. Oppositively, the liposoluble extract could incite the genes synthesis, like Aquaporin 3, Filaggrin, Involucrin, that are related to moistening.

The biologically active compound, obtained from suspension cultures of *Malus domesticus* (apple) cells, was obtained by fusion of water-soluble and fat-soluble septa, by processing the whole cell lysate with homogenization under high pressure and integrating them into thinly dispersed liposomes (nanosomes). The anti-aging activity was primarily estimated by observing the synthesis of the antioxidant enzyme Heme Oxigenase 1. *Malius domesticus* extract has an additional advantage of preventing the skin from ultraviolet irradiation [40].

The significance of phenolic antioxidants has extremely augmented recently because of their high capacity to congreagte free radicals [24]. Plants that are rich in penolics, such as *Theobroma cacao*, *Solanum tuberosum*, *Solanum lycopersicum*, *Brassica oleracea*,

Brassica oleracea, *Prunus avium*, *Citrus limon*, could be used, for example, for prevention of skin harmful effects of oxidative stress [45].

Other plants holding similar processes include *Hibiscus syriacus* (rose of Sharon) *Castanea sativa* (chestnut), *Prunus dulcis* (almond), *Juglans regia* (walnut), *Nicotiana sylvestris* (woodland tobacco) and *Quercus rubur* (oak) [46].

2.3. Purified compounds from plant cell suspension cultures

As mentioned above, plant cell cultures can act as useful biofactories for the production of industrially demanded compounds and can be induced to overproduce some types of particular substances by regulation of growth conditions, physical and chemical parameters. Crucial components of this process are elicitors, which are able to cause signaling cascades and induce the synthesis of genes that are associated with defense and the generation of secondary metabolites [49]. The compound verbascoside, a phenylpropanoid glycoside renowned for its antioxidant, anti-inflammatory, and photoprotective properties, has been synthesized in large quantities in suspension plant cell cultures of the *Buddleja davidii* (butterfly bush) [50-51]. It was demonstrated that this component suppressed the activation of pro-inflammatory agents and the action of collagenases that is related to ageing of skin [51].

Another example of a specific substance naturally produced by plant cells is trans-resveratrol, a substance that belongs to the stilbenes family and has a wide spectrum of biological activity as a medicine and in cosmetics [52]. Even though *Vitis vinifera* (grape plants) was considered the most promising source of resveratrol and its derivatives, extraction and purification on a large scale is time devouring and too expensive due to its presence in the peel but not in the fruit pulp [53]. This is why microorganisms and plant cell cultures are favored as more viable and secure sources of production [54]. To obtain a measurable amount of resveratrol in bacteria and yeast, the introduction of genes responsible for the synthesis of resveratrol into cells using genetic tools is necessary. On the contrary, suspension cultures of *V. vinifera* plant cells produce trans-resveratrol constantly and

efficiently without any genetic modification, which allows the use of various elicitors to increase the yield of resveratrol [54].

The elicitation of *Panax ginseng* and *Panax quinquefolius* (Asian and American ginseng respectively) cell suspension cultures with biotic elicitors, such as filtrates of different microbial preparations, as well as with abiotic ones, like nickel sulphate and hydrogen peroxide resulted in desired quantity of final product [53]. Ginsenosides contribute wide usage in skin care products, having bacteriostatic and bactericidal properties and are capable of inhibiting the itching on histamine-incited skin [54].

2.4. Ingredients with low content of potentially toxic compounds

Plant cell culture-derived bioactive components are free of pathogens, pollutants, and agrochemical leftovers, that as a matter of course contaminate the majority of plant-derived extracts. *In vivo*, plants usually generate venomous substances to protect themselves from exogenous pathogens and herbivores. For instance, some possibly poisonous alkaloids are synthesized by some plants as a respond to the chawing rooting from caterpillar that feed on them. Plant cell suspension cultures, cultivated in sterile environment, aren't subjected to biotic stress; consequently, there is no activation of chemical protection mechanisms. Capability to synthesize plant extracts that do not contain particular venomous alkaloids can be demonstrated by a *Lycopersicon esculentum* (tomato) extract evolved for protection of skin cells from toxic heavy metals [53, 56]. When comparing to the extracts received from fruits and leaves of tomato, the of *L. esculentum* cell cultures hydrosoluble extract doesn't consist of α-tomatine and dehydrotomatine, that are usually synthesized in almost all organs of plant as a respond to blast onslaught and are related to a couple of allergic reactions in humans [54]. Nonetheless, the obtained extract had a high number of phytochelatins, tiny metal-binding peptides that have are in charge of the metal binding capability [57], hereby it precluded decent defense from toxicity of heavy metals once catered to skin cells [54].

In similar fashion, a plant cell culture extract derived from *Coffea bengalensis* (Bengal coffee) haven't had the alkaloid caffeine and was generated as an active compound for sustaining health and homeostasis of skin. Development of a relatively new biologically

active cosmetic component that displays all the beneficial features of *Coffea* plants but not having caffeine succeeded and eventually it was able to have impact on three major kinds skin cell. The extract promoted both differentiation lipase exertion inside pre-adipocytes, enhanced synthesis of collagen I and III in the fibroblasts, and boosted the generation of genes that are in the relation with moistening inside keratinocytes, like those of the Involucrin (INV), the Filaggrin (FLG) and the Aquaporin-3 (AQP3) [52, 55].

2.5. Advantages of plant cell culture derived active ingredients

By means of a number of cell-culture derived bioactive compounds for further use in cosmetic formulation discussed in this work, it can result in emphases of their advantages. Thus, they are

- relatively simple and cost-effective,
- able to surmount the issues of manufacture in large-scale,
- capable to generate substances equal to those that already exist in the parental plant,
- have major instantaneous prospective for industrial production than differentiated cultures (cultures of tissues or organs),
- present a steady system for the permanent synthesis of secondary metabolites of stable and unvaried quality and output,
- the opportunity to produce inventive compounds that not generally synthesized by the mother plant,
- yet extracts from sparse or imperiled species of plant may become available by means plant cell culture tools,
- the effect on the ecosystem is poor, the water wastes and carbon gases imprint are decreased, and there is no requirement of pesticides or any other defoliants.

2.6. Conclusions to the chapter

Currently, a number of active ingredients developed through cell culture systems, which activity on the skin had been scientifically proved via *in vitro* and clinical studies can be seen and applied in some cosmetic formulation. Their desirable properties are provided to the cosmetic product.

As in consideration of the cosmetics industry, there is huge concernment in plant cell culture-derived extracts having composite biological properties for skin and hair care, make-up, and a hair care in the form of supplement constituents.

Plant cell culture-derived extracts that have a blend of biologically active compounds (that of not only secondary metabolites' concern) may be synthesized by now in controlled environment. It also important to note that plant cell culture-derived extracts are usually added in small concentrations to the final cosmetic products' formulation.

In addition, when estimating the advances in both cosmetic and cosmeceutical items, there must be considered the proper unification of structure of skin and functional features with the origin of the formulation, its efficiency as determined by the purpose of the component, and its security. Respectively to their effects or actions, biologically active ingredients can be divided into three main classes: moisturizing, antioxidant and anti-aging ones. Active biological compounds may diverge in their producing genesis as much as in peculiarities of their biological mechanism of action.

CHAPTER 3
ANALYSIS OF APPLIED PLANT CELL CULTURE TECHNIQUE, ITS BENEFEITS AND METHODS OF OPTIMIZATION

3.1. Advanatageous plant cell culture *modus operandi*

Because of the huge inquiries components of natural origin by non-stopping incline in human population, profitable plants are intensely synthesized on an industrial scale. Cell cultures of plants surely possess an effective substitution for the production of cosmetic biologically active components, because they always have standard, free of contaminants and biologically stable components. And hence their production can be extended to a large-scale production.

Phenolics, terpenes, flavonoids, stilbenes, steroids, saponins, terpenes, fatty acids, polysaccharides, sugars, peptides, etc. are among remarkably huge number of these phytochemicals. They can be extracted with pertinent solvents for further use as active ingredients in cosmetic formulations. Due to this, enormous numbers of plant sources have been examined by the cosmetics industry in look for novel active ingredients that could merge particular pharmacological properties. Antioxidant, antimicrobial, antiviral, anti-inflammatory, anti-allergy, as well as decent moisturizing, anti-ageing, and ultra-violet protective properties are among them and the most gainful.

Advances in plant physiology and cell biology allowed to see progress in techniques in plant cells and tissues cultures, while handling and manipulating them under sterile and regulated environment in the laboratories.

Such two features as plant plasticity and totipotency lay as a basis of plant cells and tissues manipulations. Plasticity is the ability to change metabolism of plants, while totipotency is their tissues or organs ability to regenerate from cells.

Even though various researches reporting the production of secondary metabolites using callus cultures and differentiated tissues [23, 34, 43], in most cases, undifferentiated

cells are the preferred culture system, as seen in Table 2.1. The secondary metabolites synthesis by *in vitro* cultures generally consist of further steps [3, 24]:

- the selection of potent parent material,
- an optimized surface sterilization procedure,
- the induction,
- maintenance and mass propagation of the callus culture in petri dishes,
- the initiation,
- homogenization,
- maintenance and mass propagation of the suspension culture in shake flasks and bioreactors,
- and the final cell banking of the suspension production cell line.

This current mode of synthesis may be favored by the usage undifferentiated callus, cultures of cell suspension, or even organized (differentiated) systems like roots, shoots, or somatic embryos [19].

Differentiated organ cultures are needed only then, when, for example, the desired metabolite is synthesized in only specific tissues of plant or in glands like the synthesis essential oils [10-11]. Among all differentiated systems, cultures of hairy roots present quite promising possibilities for the *in vitro* synthesis of plant-derived biologically active substances [12].

3.2. Benefits of applied plant cell culture technique

Many pluses of plant cell cultures exist as of application as founts of active ingredients accordant to plants grown in the field:

- constant supply of pure material, that does not depend on the season or the plant reproductive circuit;
- the cultivating setup can be freely regulated in order to perennially have great levels of batch to batch permanence;

– the extracted compounds are secure and pure, meaning that there is no chance of pathogens or exogenous pollution;

– the production setup is extremely manageable: no needs of rural lands, that means less water intake and farther wastes;

– adaptability, since cultures might be elicited by biochemical or genetic means: modifications in the culturing conditions, physical criterion or append of inducing substances to the culture medium can expand and enhance the levels of desired compounds' concentrations;

– in most of the cases, the extraction process is easier and less time-consuming. Besides, in case of a recombinant protein expression to a cell system culture, it may be tabbed by genetic techniques and readily secluded by uniform stage chromatography, without complicated purification staged and fractioning.

3.3. Methods for process optimization

Headways in extensive methods and inactivating approaches promote a significant rise in the quantity of plant cell cultures implementation to produce the compounds having a high appended valuation.

Commonly, secondary metabolites attribute to a smaller extent than 1% of the dry weight of plant cells, and frequently even less, hence the ameliorating the yields of longed-for metabolites is a crucial objective for their growth and enlargement. In order to increase the total outcome of desired extracts for further use in cosmetic formulations, various optimizations should take place on different stages whether cell suspension or bioreactor stage, as shown on the Figure 3.6.

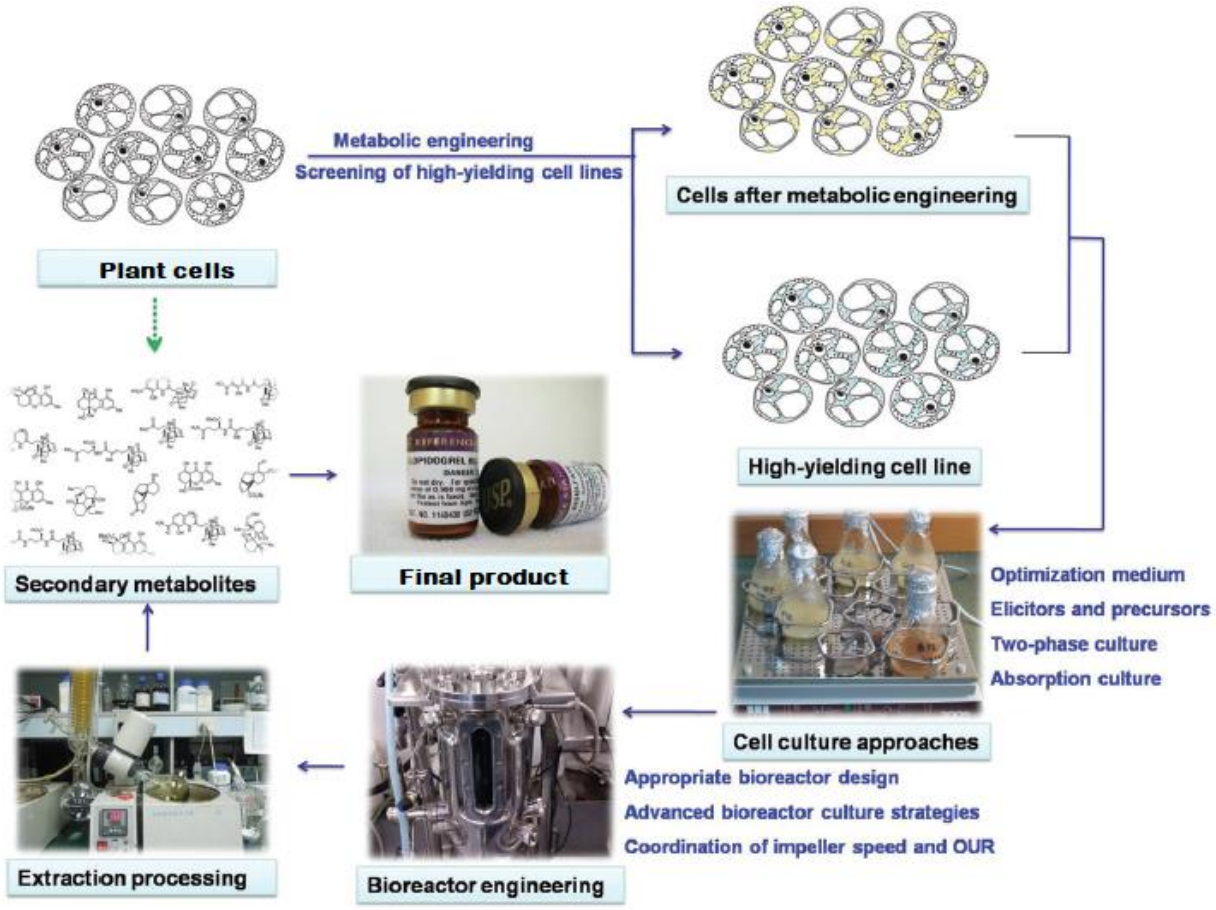

Fig. 3.6. Systematic strategies to increase secondary metabolites production with plant cell suspension cultures

Thus, these include:

– Monitoring and picking of extremely producive cell lines. Enhancement of strain starts with the selection of a parent plant having elevated portions of the longed-for products for induction of callus in order to receive extremely-producive cell lines. The choosing of extremely producive cell lines is a conventional (and vital) stage in the direction of cost-efficient manufacture, and that is because of the callus cultures heterogeneity [20]. By these means, a huge number of cells is subjected to a toxic (or cytotoxic) inhibitor or surrounding tension and only cells that can counter this stress are selected. Some examples of selective agents are p-fluorophenylalanine (PFP), phenylalanine analogue, 5-methyltryptophan, biotin and glyphosate [21].

– Media nutrients manipulations: sugar (varies from 2,5 % and up to 12% depending on the plant culture), nitrate and phosphate levels; growth regulators (the kind

and concentration of auxin or cytokinin or their proportion drastically changes the growth and the metabolite genesis in cultured plant cells); precursor feeding (e.g. phenylalanine or isocapric acid, ferulic acid, leucine or dihydroquarcetin (narigen)); or genetic engineering tools.

– Culture condition optimization: temperature (varies from 17°C up to 32°C); medium pH (extremes of pH are avoided, usually adjusted between 5 and 6 before autoclaving); agitation and aeration; air flow rate.

– Elicitation: elicitors are telltales that provoke the secondary metabolites synthesis. The substances used for process of elicitation bind to certain receptors located on the outer side of the plant cell cytomembrane and provoke signaling cascades, that turn on the transcription of genes for phytoalexins, reactive oxygen compounds and defensive enzymes synthesis. Because elicitors stimulate the synthesis of plant secondary metabolites, the procedure duration to achieve extreme concentrations of product and bigger culture bulk is reduced [20]. Elicitors can be abiotic (ultraviolet radiation, heat, cold, ions of heavy metals and various compounds, such as vanadyl sulfate, 2-hydroxyethyl jasmonate, xanthan and chitosan) and biotic (including microorganisms of fungal, bacterial and yeast origin, herbivores, and plant cell wall and membrane components, glycoproteins, or modified nucleic acids). Even though used chemical substances are nearly four to five times more expensive than the initial, their complement doesn't outstandingly heighten the general manufacture costs [22]. Features of an efficient elicitation procedure contains designation of the suitable kind of elicitor, measuring, and subjection time and, hence, is quite labor-consuming. However, elicitation is broadly used to boost the synthesis of plant cell culture-based secondary metabolites and is considered as the most efficient treatment.

– Operation mode: batch, fed-batch, continuous or perfusion.

3.4. Conclusions to the chapter

Plant cell culture technique is a reliable model system for plant science research and in vitro production of secondary metabolites for cosmeceutical use. The main advantage of

this technology is that it can be carried out under controlled conditions and various elicitors can be utilized for increasing the accumulation of the metabolites. It is independent of diverse geographical, seasonal and environmental conditions and contributes a stable production system, which ensures the continuous accumulation of products with uniform quality and yield. Numerous natural products with various activities have been obtained through this biotechnology mode.

A series of methods have also been applied to enhance the accumulation of the desired natural products. Plant cell suspension culture is still one of the most potentially useful technologies that can be utilized for the production of natural products. In addition, the research emphasis of plant cell suspension culture should be placed on:

– Study of the pharmaceutical secondary metabolites which are in short supply in clinical use, or difficult to obtain from the natural plant and hard to synthesize.

– Clarify the biosynthetic routes of natural pharmaceutical substances to exploit various methods to regulate the biosynthetic process.

– Employ membrane permeabilization, cell immobilization and *in situ* product removal techniques to improve the secretion of the desired products into the extracellular medium [22].

– Combine effective strategies such as addition of elicitors, precursors feeding, two-phase culture system and bioreactors enhance the production of desired compounds.

– Explore appropriate selection of bioreactor design and advanced bioreactor culture strategies.

CONCLUSIONS

Summing everything mentioned above, it can be easily stated that 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 and hair care as well. 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.

Many new compounds have been isolated from suspended cells of plants combined with biotransformation. The upcoming research directions of biotransformation should lie in exploring the biological activity of the new substances and enabling them to show such activities like anti-aging, anti-photo-aging anti-wrinkles, ultraviolet protection, moisturizing, soothing, toning, etc. by modifying their chemical structures.

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 intensive researches and biotechnological means needed to generate and maintain them. For vanquishing of such a restraint, advances in availability of modern biotechnological tools as well genetic means will coon take place. And with further extensive researches in field of plant cell culture to be applied, this biotechnological platform will have all the chances to be preferable in manufacture secondary metabolites having high biological activity. And thus, will able to find its deepened application in cosmetic formulations.

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