Gemmotherapy, and the Scientific Foundations of a Modern Meristemotherapy

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PREFACE

Radical planetary changes announced the advent of the 21st century, causing enormous impacts on human life. The changes were the development of previous situations already set in motion in the previous century. Several signals indicate what will occur in the future, but interpretations—and, in particular, necessary measures and counteractions—are largely lacking. The interpretation of these changes and how we act has clear consequences: the lack of this process of reflection means only being alert to something when it is already happening, i.e., not adequately considering a catastrophe or a real emergency until its occurrence. There are many examples of this attitude, and the themes and the considerations of this book are fundamental and crucial to the projections of our future. Our only hope is a general consciousness of the perils of the current pathway. Some causes of the changes can be attributed to the planet, but others are due to human influence; in any case the lack of action in the right direction will be our fault. Selected cases will here be exposed to evidence ongoing trends and the need for a new consciousness.

Acting on the current changes in the planet's climate is going to be fundamental for the success of every human activity and enterprise, from agriculture to trade. Temperature growth is generating massive migrations, from the rural to the megalopolis and from the South to the North of the planet, remodelling animal and human distribution. Technology is the key influence in these transformations, but the changes have also been fuelled by several other factors: the crisis of the traditional family model, urbanization, and progressive dependence on artificial intelligence and the global connection network. However, it's probable that current changes are mainly connected with the increase in life expectancy, with the emergence of new pathologies and health disorders, and the revolution in the nutritional environment, due to radical changes in food. Changes are rapidly affecting our quality of life and health, asking for new approaches and solutions. If we want to imagine the future, as derived from this "evolution sap", we must consider alternative utilisations of natural resources, facing the challenges of sustainability and overconsumption. The adequate utilisation of plant remedies is useful for inverting the tendency of dramatically exploiting any of the planet's resources.

This book is focused on gemmotherapy—also reported as meristemotherapy or phytoembryotherapy—meaning a medical treatment that uses remedies principally obtained from the meristem raw material of various juvenile tissues, like shrubs, emerging shoots, buds, cambium, even seeds and young roots. Among gemmotherapy topics, we find the production of bud-derivatives and validation of their utilisation.

The main concern about the future of gemmotherapy is a part of the general debate on the rational and coherent utilisation of natural resources. Its dedicated target is the satisfaction of the great human desire for a long life, free from disease and sickness, by using a modern and natural approach. In fact, new tendencies and new products are emerging, and they are the result of the careful utilisation of natural products obtained from novel raw materials transformed into marketed offers, which are useful for healthcare and wellbeing.

This new use (in addition to other natural approaches in medical treatment) has caused much surprise, as for a long time natural substances have been relegated to an ancillary role. Natural products are experiencing a new renaissance after a long period of difficulties when they were considered mainly as models or templates for the development of new pharmaceutical drugs. Natural resources, like buds and shoots, can be considered as a natural means of living in harmony with our body and the environment. Gemmotherapy is a part of this movement. The aim of this book is to report the current state of this discipline and the next steps, in order to inform everyone interested, starting with those working in medicinal sectors, how to appreciate and utilise the potentiality of buds in a reliable and complete way. The target of this book is every interested person, including the student and the curious, the producer and the consumer.

Nowadays, natural products are regaining their principal role in health maintenance with an explosion of marketed solutions in food supplements and phytomedicine. Areas of this sector are evolving fast and continuously creating information, claims, rules, and quality controls. The result is complicated, but also widespread and fascinating. This is evident particularly in gemmotherapy, where the current sources of information are very few, though the use of plant meristems is greatly increasing.

This book intends to fill this gap, offering a complete update of the gemmotherapy world.

PART I

CHAPTER 1

NEW FRONTIERS FOR NATURAL PRODUCTS

Introduction to Chapter 1

This chapter concerns the recent utilisation of natural plant substances, from food supplements to bud-derivatives. In the market of new fast-growing areas of herbal products, recent introductions have exerted a revolutionary impact on the concept of food and its ordinary range.

Herbal product use, starting from a generic request for the "natural", has been subjected to several variations, which have modified its limits and inputs:

a) Scientific knowledge. Thanks to the enormous work of phytochemistry, we can rely on an ample catalogue of natural products, based on hundreds of thousands of identified and determined structures, which are today very useful as standards and references of data (not to mention another four hundred thousand similar products synthesised by the chemical industry).
b) The appearance on the market of new products for health needs. A great quantity of marketed products is accessible in accordance with emerging requests for a better life.
c) Quality control. Nowadays, raw plant materials are subjected to several checking stages and radical transformations until they become marketed final products. Therefore, proper controls on their chemical constitution are necessary to ensure efficacy and security.
d) Recently, new fast-growing areas have exerted a revolutionary impact on the food supplement market. The new products, generally called nutraceuticals, offer health benefits and have a positive effect on metabolic disorders. The discovery, development, and marketing of these products have been fuelled by their positive reception by consumers. The nutritional sphere is at the centre of everybody's attention, from governments to ordinary people. Besides the omnipresent concerns regarding the quantity of food, recently quality has become fundamental for maintaining health and

improvement in lifestyle.

The main categories of food supplements can be organized on the basis of their appearance on the market:

First Generation	Vitamins, Proteins, Minerals, Probiotics
Second Generation	Nutraceuticals, Botanicals, Novel foods
Third generation	Functional foods, Multifunctional foods
Future generation	Bud-derivatives, Cosmeceuticals, Pharmafoods

The differences between these categories lie in form and composition (Fig. 1). The constituents of the first-generation food supplements were limited to vitamins, minerals, proteins, and carbohydrates, mainly addressing dietary and other simple targets. Their explicit goals were limited to supporting nutritional needs and/or supplying food deficiencies.

Later, a second generation of food supplements appeared, subsequent to increasing expectations for a better life and health maintenance, with the consequent contamination of the narrow line between drug and food. Their composition showed a massive introduction of natural products, collectively named “other substances”, which distinguished them from the former constituents. Other substances were mainly extracts of medicinal plants, meaning the same ones used for centuries as remedies in the ethnobotanical herbal tradition, but often also as foods or spices. However, the identity of these products (in composition, utilisation, manufacture, quality control, etc.) were different from those of the first generation. Therefore, several new names spontaneously appeared to mark the differences, including nutraceuticals (the most frequently used, but still not officially recognised), dietary supplements, medical remedies, herbal drug preparations, traditional herbal medicine products, and others. Recently, botanical food supplements, or botanical supplements, or simply botanicals, are the words used to name food supplements, which are derived only from plant raw materials.

It is time to empower the third generation of food supplements. The future invasion of the food market will see the appearance of special medical or health foods, or simply pharmafoods, that will be followed by functional foods, fortified foods, and multifunctional foods. Marketing creativity has even produced superfoods. Among them, the introduction of new raw materials, like buds, is relevant.

Nutraceuticals propose health benefits and positive effects in case of metabolic disorders. The nutritional environment is at the center of

everybody's attention, from governments to ordinary people. Besides the eternal problems concerning the quantity of food, recently quality has become fundamental for health maintenance and lifestyle improvements. Quality involves the effects of the composition of food on health, and the need to integrate or improve the effects of the ordinary food.

The result is a complex dynamic situation, requiring careful analysis of each of the various aspects. Only people directly involved in this matter can understand the process and explain the complicated mechanisms. Furthermore, besides the official documents, there are few sources of information explaining all aspects of the sector. Usually, information is scattered in single arguments or is available only to specialised people.

The common aspect in natural products is the presence of nutrients, in contrast with medicinal drugs, where synthetic products are prelevant and generally considered dominant. In the last few decades, food supplements have undergone a complex evolution that has radically changed their forms, targets, and composition, influencing health aspects at the same time (Fig. 1).

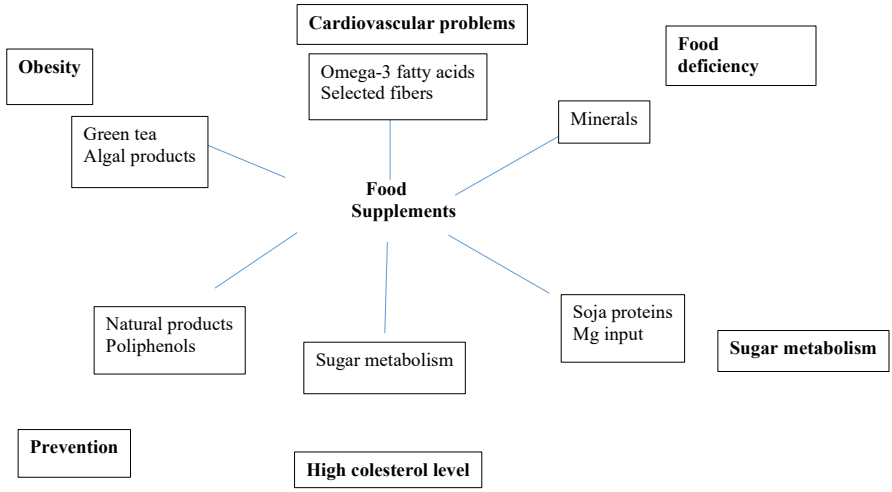

Fig. 1. The large and complex situation of new marketed products based on natural products, in relation with their pharmacologic and clinic utilizations.

The food supplements galaxy

Definition of food supplements by UE Directive 2002 – 2002/46/CE:

> Food supplements are "*concentrated sources of nutrients with nutritional of physiological effects whose purpose is to supplement the ordinary diet, they are designed to supply nutrients, micronutrients and other physiologically active substances in predetermined amounts. They are marketed in dose form, i.e. pills, tablets, capsules, liquids, etc. in a measured dose*."

Definition of Dietary Food by the Food and Drug Administration (USA)

> "*Conventional foods are foods that are not dietary supplements. A dietary supplement is a product taken by mouth that is intended to supplement the diet and that contains one or more "dietary ingredients." The "dietary ingredients" in these products may include: vitamins, minerals, herbs or other botanicals, amino acids, other substances found in the human diet, such as enzymes*."

Definition of a Nutraceutical by the inventor Dr. Stephen DeFelice:

> "*Food, or parts of food, that provide medical or health benefits, including the prevention and treatment of disease*."

Definition of a Nutraceutical by the Oxford Dictionary:

> *"A food containing health-giving additives and having medicinal benefit"*.

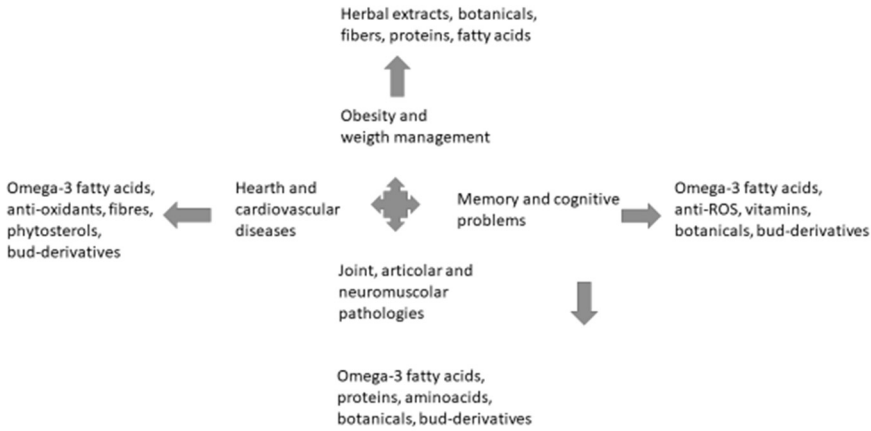

Fig. 2. Expectations of consumers regarding a better life and nutraceuticals ingredients.

The term functional food was first officially introduced in Japan in the mid-1980s, defined as FOSHU, an acronym meaning FOods for Specific Health Use. These foods are eligible to bear a seal of approval from the Japanese Ministry of Health and Welfare. In Japan, more than 100 products are licensed as FOSHU. So far in Europe, the functional food category is not legally recognised.

The effectiveness of a FOSHU product on the human body is proven by several characters:

* Absence of any safety issues (animal toxicity tests, confirmation of effects in the cases of excess intake, etc.)
* Use of nutritionally appropriate ingredients (e.g. no excessive use of salt, etc.)
* Guarantee of compatibility with product specifications by the time of consumption
* Established quality control methods, such as specifications of products and ingredients, processes, and methods of analysis. For example, dietary fibre, sugar alcohols, oligosaccharides, proteins, polyphenols, lacto- or bifido-bacilli, chitosan, and sodium alginate are considered helpful in maintaining good health.

The food supplements front is completed by the products related to microorganisms, concerning probiotics, the symbiotic bacteria, and prebiotics, which are useful for their health and survival. Here, we will focus on emerging products, like bud-derivatives (Fig. 2).

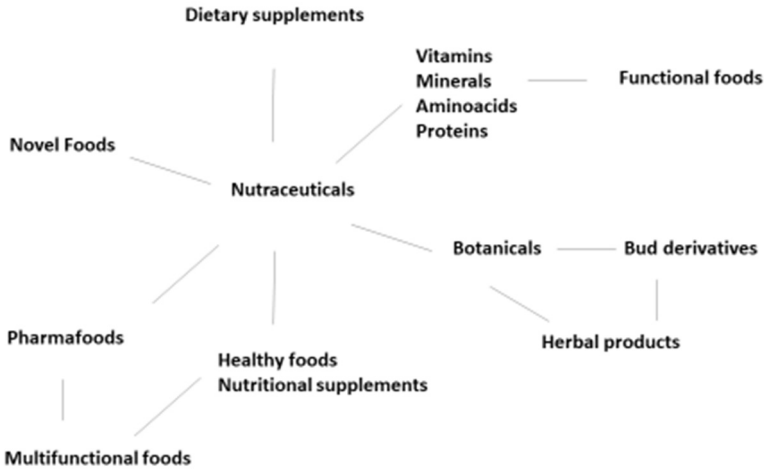

Fig. 3. The galaxy of food supplements and nutraceuticals.

Evolution of food supplements

Nutraceuticals imposed their importance by the number of them on the market. That is, from their appearance these numbers increased constantly, both in money and in sales. During the last decade, this impact attracted the general attention of ordinary people and producers, despite the connected problems of safety, massive regulation, and compliance (Fig. 3). At the same time, the regulation about these products generated a plethora of indications, responsible of an increase of the confusion about the food supplements (Fig. 4).

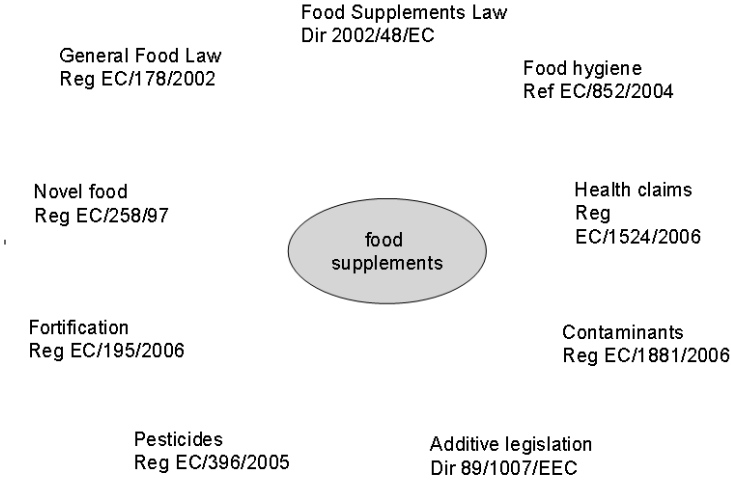

Fig. 4. The relevant figure of Regulations in E.U. concerning food supplements.

Fig. 5. The market of US Nutraceutical Market and the expected growing.

The food supplement market shows an upward tendency (Fig. 5), which is confirmed for the entire sector of natural products. The nutraceutical market, including pre-biotics and pro-biotics, vitamins, minerals, fibres, proteins, omega-3 and structured lipids, amino acids, functional foods, and varied forms of dietary supplements, is consolidated and improving. The amount of sales involved in all sectors reached $142.1 billion in 2011, was valued at around $250 billion in 2014, and is expected to reach $204.8 billion by 2017 and 385 billion by 2020, with a growing rate at a compound annual growth rate (CAGR) of 6.3-7.5%. The nutraceutical foods fortified with omega fatty acids are expected to grow by 6.8% during the forecast period. The nations spearheading the global nutraceutical markets are the United States, Japan, Israel, and Germany. Rising consumption of dietary supplements among the baby boomer generation and the thriving food and beverages market is driving the US nutraceutical market, which is expected to reach $90 billion by 2020, at an increase of 6%. The United States should also dominate the functional food market globally by 2020, with an expected 8.8%. This is followed by Asia-Pacific: 47% of the Japanese population consumes nutraceutical products, probably thanks to a growing ageing population.

The US market is considered more reliable, large, and active, but the EU market is more differentiated, dynamic, and is progressing rapidly. Nowadays, within the EU market, vitamins and minerals hold the largest share (about 50-55%: €4.1 - €4.73 billion), with the balance (€3.87 - €4.1 billion) constituted by “other substances”, mainly containing extracts of plants and their constituents. In 2005, Euromonitor estimated that probiotics accounted for 45% of the EU market, followed by combination products at 44%, including all the “other substances” food supplements. It is estimated all the other product categories accounted for less than 10% of the retail value. Several EU companies believe that innovation is the way forward for the nutraceutical industry: in particular, Germany, the Netherlands, and Sweden have emerged as nutraceutical innovation hubs in Europe, while Great Britain and Spain have emerged as key test markets for new products. Italy is the leader in Europe with one third of the Italian population consuming dietary supplements regularly, in accordance with a strong herbal tradition. US companies are above all looking to diversify their products and are leaning towards natural nutraceutical ingredients in them, mainly following the increasing demand for all-natural, non-modified functional ingredients.

Usually the reasons for the success of natural products are related not only to a propensity towards “naturalness”, converted into a generic request for

biological products, but also towards other more inherent matters.

- The ageing of the world population and expectations of life are growing exponentially. In Europe, people aged over 65 are expected to double in 2060 and reach nearly 30% of the total population in 2080. Roughly 8% of the worldwide population, about 500 million, are over the age of 65. Individuals over 65 will number more than 1 billion in 2020, among them 70% living in developed countries and having the benefit of a good spending capacity. However, rapid increases are expected among the elderly in developing countries. The main preoccupation of all these people will be to maintain good health conditions by requesting anti-ageing and weight control products. The general tendency is to avoid chemical drugs and medical controls. Improved diagnostic methods are leading to the earlier diagnosis of diseases or simple dysfunctions. Patients increasingly wish to control and manage directly their conditions and are incredibly alert with regard to increasing cholesterol levels or weight diagnostics. The next step for these people is a continuous collection of information, either by direct acquisition or by following the sirens of marketing. These new types of self-made health-conscious consumers are the strong and durable basis of the nutraceuticals market.
- Cuts in healthcare costs will lead governments to increase self-diagnosis and self-medication. In this kind of situation, marketing is highly facilitated, convincing people with all sorts of product promotion and offering spectacular results. The trend is already consolidated, nutraceuticals being utilised to increase the strong over the counter (OTC) market. Easy production conditions and limited controls are also attracting the interest of the big pharmaceutical companies.
- Domestic growth of nutraceutical consumption is expected to continue and increase, fueled by the appearance of new attractive products and new segments targeted by local producers for domestic requirements.
- The nutraceuticals market is rapidly expanding towards unexplored targets, like pet care and livestock feed. The future expectations of this market are impressive and practically without limits. The importance of nutraceuticals will be higher if today's multidrug resistance (MDR) continues, especially in the field of antibacterial medication. Antibiotic resistance is rising to dangerously high levels in all parts of the world. Several studies are prospecting the end of the Antibiotic Age, meaning that soon most of the current

antibacterial drugs will have no effect against microorganisms' attacks. We are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill. Other strategies must be experimented, again exploring mother nature's molecular gifts, including prevention and reinforcing the immune system's capacities.

- These are only a few of the possible arguments (and others can be presented), but probably the most convincing causes of the boom can be found in the crisis within the pharmaceutical industry, strangled by the overwhelming cost of adverse claims and the evidence of long term collateral effects. Furthermore, the expectations of a healthier, longer lifespan are not really a pharmaceutical matter, pharmacology having been focused up until now on the treatment of evident pathologies. The care of people's health in order to improve their physiological welfare is being—and in time will have been—efficiently assumed by nutraceuticals. Thus, the gap created in the market and in consumers' perceptions between medical drugs and food has been promptly filled by nutraceuticals, which are the modern answer to the new request and increasing expectation of a better and healthier life.
- Up until today, food supplements and related products have benefited from relative freedom or indulgence from restraints and easy marketing. However, this scenario could be radically changed by the introduction of the claims regime for food supplements. In this case, any declared influence on a disease, or a pathological state, must be justified at the same level as a medicinal drug. In the EU, the toxicity of nutraceuticals, as well as adherence to their claims, has been seriously considered by the EFSA (European Food Safety Authority), practically adopting the same judgment criteria used for medical drugs. Consequently, the first examination resulted in the rejection of almost all botanical claims (c. 95%). Based on the current judgment criteria, the situation will not improve, causing the exclusion from the market of most botanicals. The future impact on the market of EFSA statements has been considered devastating. The economic impact assessment of the EU Regulation on Nutrition and Health Claims (EC/1924/2006) on the EU food supplements sector and market of botanicals has been calculated in an independent study commissioned by the European Health Claims Alliance. The effects of adoption of legal decisions proposed by EFSA will affect the majority of the deposited claims, resulting in a decrease in size by about 25% of the whole sector. In two years, one third of the sector

could be at risk. The subsequent fall in employment is expected to be of about 18% of the total employment in botanicals and "other substances". In 2009, the employment in the "other substances" sector accounted for an estimated 70,500 people (44,930 units in the major producer countries). The negative economic impasse, increased by the claims' impeachment effects, could have a great impact on the European food supplement industry and its employment. On the other hand, the simple introduction of nutraceuticals into the old category of food has generated a continuous conflict with their non-nutritive character.

- Among the arguments of the debate opposing the simple identification of nutraceuticals with food, we can find posited their retail points, marketing information, the adopted drug form, their composition, the target consumer, and the consumers' expectations. On the other hand, in favour of the food categorisation, there is: the absence of quantification of the constituents; the activity of physiological functions and the lack or vagueness of target diseases; difficulties in identifying the active constituents; and their similarity to other food products, like juices, yogurts, hydrocolloids, etc. In reality, all these aspects can be summarised in the novelty of this kind of product, which cannot be considered either as food or as a drug. Therefore, these new products should appeal for adequate protocols in accordance with their special nature. The multi-millionaire nutraceuticals industry has to face this problem and invest part of the accumulated money into research converting pharmaceutical and clinical studies into adapted innovative knowledge. The nutraceuticals and botanicals market in the near future is probably going to experience a complicated period without any security apart from the considerable amount of interest and money involved. This is the real insurance card for the survival of these products while they await scientific support.

On the basis of the aforementioned background, bud-derivatives can now be assigned to the world of botanicals/herbal medicinal products. However, several peculiar characteristics have to be considered:

a) The presence of meristems and juvenile tissues, whereas mature materials are preferred in botanicals
b) The raw material usually consisting in the fresh, i.e. recently collected, part of a plant
c) The extraction process involving glycerol and ethyl alcohol
d) The therapeutic approach based on the original intuitions of Pol

Henry and confirmed by clinical experience and modern techniques
e) The different chemical composition, as fully reported for the first time in this book.

In conclusion, gemmotherapy must be considered as a special case among the therapeutic tools based on natural products. Its evolution is strictly related to the galaxy of the new marketed products generated by the request for health by natural approaches. Before considering its efficacy and utility, it is necessary to understand its peculiar nature and acquire full knowledge of its potential.

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CHAPTER 2

THE GREEN LIVING WORLD

Introduction to Chapter 2

Plants are exceptional examples of adaptation to environmental conditions. We are not able to fully comprehend their ability to change, being more accustomed to variations in animals, like the enormous neck of a giraffe or the wings of a bat. However, sometimes evidence of adaptation in animals is also difficult to understand, for example the change in a butterfly's colours caused by pollution. Adaptation changes in plants are very important and impressive only when we accommodate our sensibility to plant metabolism. Plants act in silence, slowly, with respect for the environment. We must consider that they have had much more time to adopt strategies and develop solutions, and therefore we have a lot to learn from their abilities.

Every year, in harmony with the seasonal changes, plants are able to react to temperature, moisture, and wind, by modifying their morphology and metabolism. Certainly, in temperate zones, spring is the most spectacular and astonishing time, when trees experience a real revolution in their structural and molecular organisation. The waking-up of vegetation after a long and difficult period of stasis, when for months the trees appeared dead and immobile, demonstrates an extreme capacity for renaissance. Several questions naturally arise. What is moving inside their bodies? What kind of force animates the buds to generate branches and leaves? How is a tiny seed able to start on the pathway to becoming a magnificent oak or a powerful giant sequoia? However, more importantly, is it possible to grasp this prodigious capacity and use it for medicinal purposes?

Plant meristems

The key of the extraordinary plant exploits is the presence of particular cells, totipotent cells, named meristem (divisible, divide into parts) for their capacity to operate continuously the miracle of mitosis, modulating cell

generation and reacting properly every time. New cells must act in relation to the environmental situation, but in accordance with the genome strategies. In the meantime, the plant's juvenile cell reacts to the habitat pressure and maintains intact the general project of programmed development of the species. Inside the plants, there are many microscopic "seeds", like embryotic cells, distributed throughout several parts of the plant; these are active in all plant life. We are talking of very special cells, capable of the supreme denial, being able to face apoptosis, i.e. programmed death, which is the torment of each living being from the moment of birth.

The expectation is that the same vitality can in some way be captured and technologically transferred. We can not remain indifferent to the miracles of plant revival, and we have to try to obtain the essence of buds and shoots, with the help of scientific knowledge and technological devices. An opportunity is now available, after centuries of inadequate and thwarted attempts. The challenge of modern meristemotherapy is the revelation of the most intimate secrets of plants, to make them available for the benefit and health of humankind. It is evident that this tentative ambition is in the tradition of phytotherapy.

A parallel approach can be proposed with staminal cells, although it is clear that we must go a long way in order to obtain an effective utilisation of staminal cells, even if we do not take into consideration the possible barriers caused by ethical questions. While waiting for answers to overcome questions and doubts, some easier applications have started in the field of plant science, where ethical arguments are so far minimal. Utilisations in gemmotherapy are focused on juvenile tissues, which can be considered the staminal cells of plants. Plant staminal cells are called meristems. Despite the many analogies, several differences between juvenile tissues of animals and plant must be considered. The main difference between staminal cells and plant meristems is that meristems are active during all the steps of plant life and distributed throughout several parts of the plant's body. Some applications for them have already been realised. Plant micropropagation using juvenile plant cells is widely used and its methods are applied in many cases for selection of cultivars and reproduction in sterile conditions.

Vascular plant structure is based on three basic mature tissue types (Fig. 6): ground tissue (parenchyma, sclerenchyma and collenchyma), vascular tissue (phloem, xylem), and dermal tissue (epidermis, endodermis, bark). All of these tissues arise from specific meristems, consisting of small regions of undifferentiated cells, in which most cell divisions take place (Fig. 7). Two systems, responsible for the plant's architecture, are present:

the root system and the shoot system. The root system consists of branching roots, which anchor the plant and penetrate the soil, to absorb water and ions, but also to integrate the plant with the soil network. The shoot system consists of the stem branches and their leaves sprouting. Stems and roots have apical meristems that produce the primary growth of the plant, whereas the secondary growth, involving wood or bark, arises from the cambium, a later meristem layer. The vascular cambium is a ring of cells, one layer thick, that produces tissues, like secondary xylem and secondary phloem, as well as parenchymal cells in the stem rays. In plants growing in temperate regions, the activity of meristems is concentrated in the vegetative shoots, usually in axillary positions. There is an iterative unit of the vegetative shoot, consisting of the internode, node leaf and axillary bud. In terms of a tree's growth, the axillary meristems are considered lateral, in consideration of their development, whereas the apical ones are considered vertical. These meristems are the evidence of the different organisation of plants compared to animals. Animal organisations are models of centralised systems based on brain activity, whereas in plants everything is delocalised in a plurality of units working together in a network. Both plurality against the concentration of activity and buds are responsible for the development of the plant in accordance with the genome of the species. The key role of meristems is also important for their correct utilisation in meristemotherapy.

The term meristem was coined in 1858 by Carl Wilhelm von Nageli, referring to types of plant cells, which are able to give rise to entire organs. In the modern definition, a meristem is considered a population of mitotically active cells able to produce new cells, tissues, or organs in plants, excluding small clones potentially active for reproduction. Much of the adult plant body is patterned post embryonically through the action of coordinated groups of meristem cells in the stem apex, root apices, and shoots. From the germinating seed to the mature stage, the development of the plant body depends on the activity of meristems.

Plant meristems consist of clumps of small totipotent cells, characterised by a dense cytoplasm and large nucleus. Meristems are mainly present in shoots, buds, and apices, as well as in other parts of the plant. However, the definitive parenchymal cells are able to work in a similar way if necessary. The function of meristem cells consists in the generation of new cells that will differentiate accordingly to their position and final function. Each cell of the meristem divides in mitosis to give rise to two cells. One of them remains meristematic, while the other undergoes differentiation according to its role inside the plant body. That is, in the meristematic area there are juvenile cells, working so that the population of meristem cells is

continually renewed and a series of cells are produced. Meristems are divided into two types: a) the primary, mainly derived from the embryo, which are responsible for the tissue architecture of herbs and for the primary structure of any plant; and b) the secondary, which, when present, contains the tissue from which the production of plant secondary structure derives, including most wood and additional functional parts.

In the meristem, the produced cells, at first indeterminate, follow their destiny until maturity, achieving different shapes and displaying different activities in accordance with their position in the pertinent organ. Thanks to meristems, the plant grows, changes, and renews itself. Even when the plant is not growing, the meristems are still present and active. Thanks to these cells, in temperate zones each year in spring, perennial plants restart their lives, generating new tissues, since meristems elaborate the body of plants according to the environmental conditions and the plant's development plan.

Apical meristems produce the primary tissues forming the so-called primary plant body. Both root and shoot apical meristems are composed of little delicate cells. In particular, the root apical meristems, moving through the soil, must be protected by the root cap. Many herbaceous plants undergo only primary growth, whereas others, like woody plants, experience a secondary growth, which is accomplished mainly by lateral meristems. Tissues formed by these new meristems are responsible for most of the plant body, including the trunk, branches, older roots of trees, and shrubs, and are collectively called the secondary plant body, compared to the primary one now limited to its innermost parts and derived from the first step of plant growth from the plantula.

The potential of meristems has not been ignored by the nutraceuticals sector. Among botanicals, the most innovative products, like those utilised in meristemotherapy, deserve special attention. The utilisation of meristems possesses a special appeal, derived from the therapeutic use of buds or other young and developing vegetal parts. The general idea is to profit from the renewing force of growing or of newborn parts of the plant. Nowadays, this is reported in the new approach based on botanicals focused on the use of juvenile parts, named in several ways, e.g. bud-, meristematic-, embryo-, phytoembryo-, or blasto-therapy, among others. For the sake of simplicity, we will adopt the terms gemmotherapy or meristemotherapy, referring to a modern non-conventional medical therapy, using plant raw materials rich in meristems to obtain bud-derived products. Despite an increase in public interest and requests, gemmotherapy is still in search of its proper definition,

recognition, validation, and an adequate regulatory acceptance.

The true meristem is a special very narrow membrane, one cell thick. Therefore, only experts in plant histology are able to separate the meristem from the adjacent cells. The result is that, usually inside the bud, we find a mix of cells, which are very different in shape and function, even in the differentiation stage. This is a key consideration with regard to other reports, where meristems are considered a distinct tissue easy to detect and separate, where the only distinctive character is the function. Inside a bud, there is a mix of cells at different stages of differentiation and with different positions, including the double one in part producing other cells and in part remaining juvenile. In the production of bud-derivatives, the raw material is restricted to fresh plant tissues or juvenile parts, like buds, shoots, young roots, and branchlets and their bark, as well as any other tissues in some way containing meristems or developing cells. In the presence of the embryo, seeds and fruits are included too.

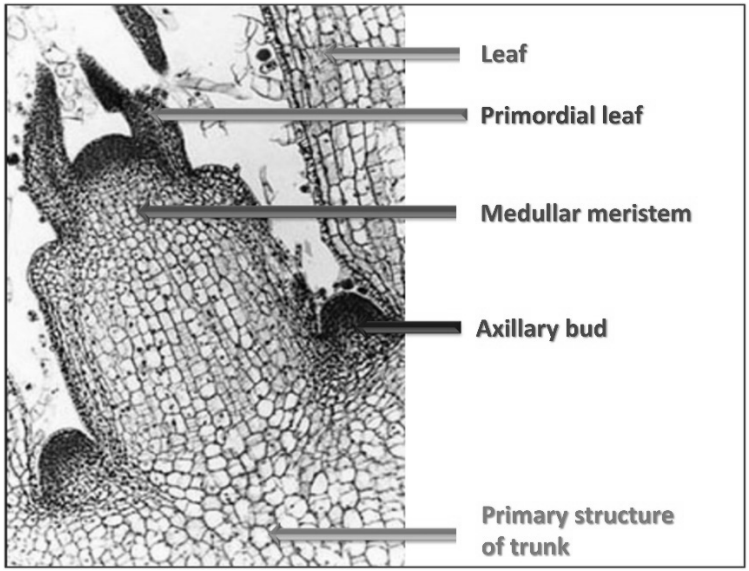

Fig. 6. Localization of meristems in apical, axillar, and root apex buds. Apical bud, axillary buss, stem, root, little root, root apex.

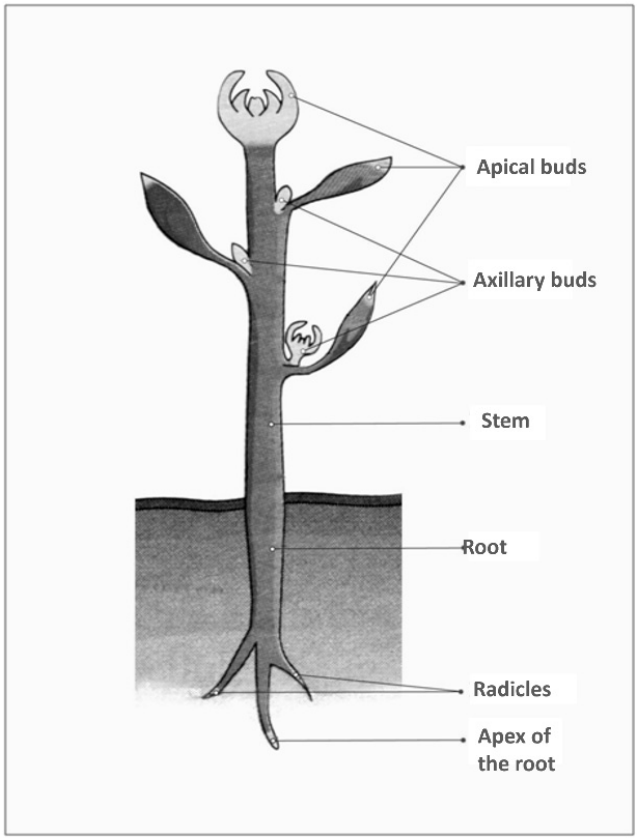

Fig. 7. Vegetative apex and other meristematic structures, including the foliar and branch primordia, present at the beginning as simple little axillary appendices developing in buds.

Development of meristems

To understand the development of a meristem, we must consider that in a short time during the spring, buds are able to regenerate the new latent tree. This is the result of a process, which started several weeks earlier. Inside the vegetative apex and in the axillary regions, the production of primordia of the leaves and branches by the bud has been going on for a long time. During this time, the primordia are invisible, because they are deep inside and protected by the perulas (or perules). Perulas are scales covering a bud,

consisting of specialized leaves metamorphosed exactly for this role. The aim of the meristems is the production of cells, but the speed of this production is slowed down for long time, a pit stop in the construction of the future architecture of new parts, awaiting better times to accelerate. The acceleration is possible in consideration of this precise work. When the temperatures rise, rainfall gives an enormous quantity of water and the longer days provide the necessary sunlight; the buds are then ready for metamorphosis. The concentrated meristem layers produce differentiated cells in quantity, resulting in the change of the buds into shoots. The young shoots have already produced myriads of little young leaves and branches, which are much bigger than buds and therefore are now visible. The passage from the bud to the shoot is generally rapid; the plant has already taken the long awaited pathway and is going to develop all its capacity for growing. The mature shoot is already the model of a little plant, where the meristems are now limited although still active. Inside the shoot, the new cells are of sufficient number and they have only to grow in accordance with their position inside the bud. The final step is a natural evolution towards the final formation of the organs. The difference between an apical and a lateral (axillary) bud is mainly due to the number of primordia, the model being essentially the same and corresponding to that which is called a fractal in mathematics.

In conclusion, we have the sequence bud > young shoot > mature shoots > generated organs, with a progressive decrease in the percentage of undifferentiated cells (meristems) in favour of the differentiated cells, pertaining to one of the aforementioned types of tissues (Fig. 8).

Buds and shoots, as in any meristem, are the unique result of a balance. They are never static nor homogeneous. They are the plant's dynamic reply to environmental challenges. A balance between cell division and differentiation, cell wall loosening and wall tightening, they work as a mixture of maintenance and change, stability and growth, life and death, all forced to coexist, until the new cells claim their final supremacy.

Therefore, in plants, all tissues and organs—albeit very different in form, shape and function—are the result of the development of a unique starting model: the bud, including the specialised meristems. The representation of such a process resembles the phylogenetic tree, where, starting from an initial common ancestor, the descendants are the result of a progressive process of dissemination with an increasing of number of taxa and a decreasing difference between taxa of the same group (clade). A phylogenetic tree models the phylogeny of an observed set of species (taxa)

and of their unobserved common ancestors. Its terminal spots (leaves) represent the observed taxa, its internal vertices represent the common ancestors, and each edge weight represents the evolutionary distance between the pair of vertices connected by the associated edge. We really do not know if reality is in accordance with this approach, named monophyletic, but its application has been very useful for obtaining interpretations of natural phenomena or the classification of complex systems in biology. Typical monophyletic trees come from molecular biology. We can consider a case involving all of us, like the origin of mammals. At the time of the decline of the dinosaurs, from Monotremata, only two groups were generated, Marsupalia and Placentalia, each of them producing several subgroups, always in accordance with the monophyletic scheme. Following the phylogenetic tree, we find out that all marine mammals share a common ancestor (still unknown) and our close ancestor was probably a lemur. This approach allows a simplification of the complexity of the reality and can be successfully applied in many fields. Therefore, in phytochemistry, all the isoquinoline alkaloids come from the reticuline structure generated from the junction of two phenylalanine moieties and all monoterpenes have the geraniol as their unique precursor. In any case, botany and chemistry must coincide strictly. Throughout a period of 50 years, based on an evolution in applied experiments and clinical trials, this method evolved in terms of research and the validation of its points of view. Therefore, thanks to the network involving any living organism and those that lived before, the buds can be considered as a key to understanding some of the secrets of life.

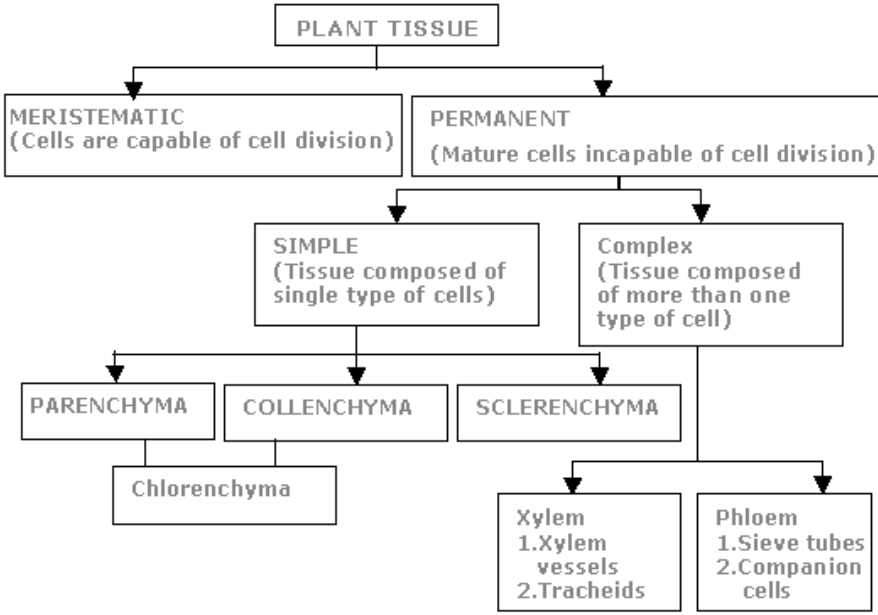

Fig. 8. Main differentiated structures in plant body.