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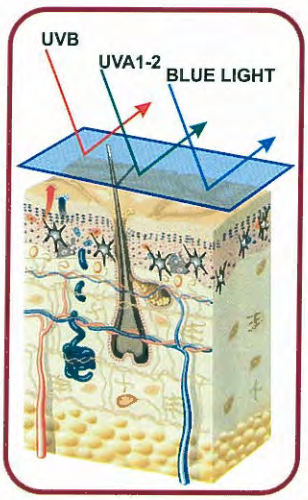

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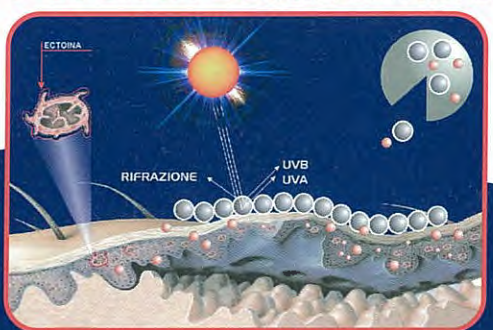

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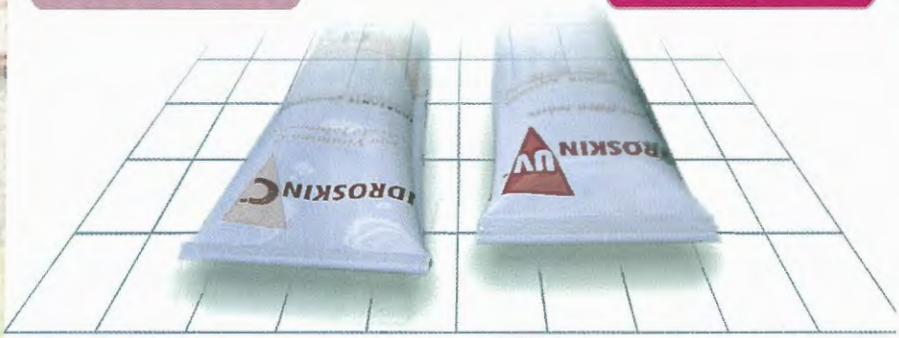

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West Cosmetology is looking to East: the NICE and TMC concepts

A growing demand for customization and personalization, driving the development of new kind of cosmetic products (cosmeceuticals and nutricosmetics), requires new kind of customer information. It means that consumer demand is more important than ever, and it calls for new and more effective products giving not only beauty but a general wellbeing.

Thus, an affluent woman who prefers high fashion outfits and affluent woman who prefers conservative luxury can be motivated by entirely different things, even though they both bought the same lipstick. They are looking for products capable to give to the whole body a healthy state and wellbeing sensation.

As consumers are getting more and more sophisticated technology, products are becoming more and more sophisticated too. As customers become more important and more demanding, researchers and market department are seeking to build stronger relationship with them.

Thus, the so called, neurocosmetics are entering the market.

Based on the NICE approach, where, nervous, immune, cutaneous and endocrine systems work together to give beauty and wellness, cosmeceuticals have to possess characteristics combining the aesthetic appeal and benefits of traditional cosmetic products with therapeutic components. Neurocosmetics have to be capable to coordinate the intercellular signals coming from the immune and the endocrine systems, integrating them with the biological signals and activities of the skin, connected by the brain neuromediators.

This new approach to formulate and control the cosmetic activity taken both from inside to outside for obtaining a general efficacy at level of the entire body, go in the same direction of the Traditional Chinese Medicine (TMC) that take the body as an organic whole.

Philosophy in ancient China believed that the universe and the human body were made of yin and yang, the constant movement of which was responsible for the existence of the world and the wellness of the body. The alternation of ying and yang was taken as the low of the everything, the balance between ying and yang was thought as the ideal state.

The purpose of neurocosmetics based on the NICE approach is to obtain beauty and wellness of our body in the same way as the therapeutic methods of TMC like to adjust the state of ying and yang to the harmonious level.

Some selected articles reported in this issue should contribute for a better understanding of this new cathegory of cosmetic products called neurocosmetics.

P. Morganti
Editor-in-Chief

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Cell Management for Innovative Cosmetics: the NICE-TMC Approach

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Summary

The skin is a multicellular organ comprising different cells necessary for responding to intracellular and extracellular signals coming from the interior and exterior environment.
This cell communication network appears essential to regulate its own life and development acting as a biologic regulatory language by the use of peptide messengers.
These macromolecular peptides represent the biochemical expression to coordinate the immune and endocrine systems linking each other to the biological activities of the skin by the nervous system.
For all these reasons, at level of cosmeceutical formulations, the NICE (Nervous, Immune, Cutaneous, Endocrine) approach has been developed to experimentally demonstrate how these four systems work all together to give us the desired global wellness and beauty.
This is the future challenge in the actual global and transforming society, where health and beauty concern become the ongoing goals for an ageing population.

Riassunto

La cute è un organo multicellulare organizzato con diverse tipologie di cellule necessarie per interpretare i molteplici segnali provenienti dall'ambiente sia esterno che interno dal quale è circondato. Questa struttura di comunicazione risulta fondamentale per regolare lo sviluppo e la vita cellulare attraverso il linguaggio biologico interpretato dai peptidi messaggeri. Questi peptidi traducono in messaggi l'espressione biochimica delle emozioni e svolgono un ruolo fondamentale nel coordinare tutte le attività svolte dai sistemi endocrino ed immune collegandoli entrambi alle attività biologiche della cute attraverso l'azione del sistema nervoso. Per tutti questi motivi è stato sviluppato l'approccio formulativo NICE per dimostrare come l'uso di particolari cosmetici appositamente formulati potrebbero darci il benessere e la bellezza globale a cui aspiriamo, attraverso l'attivazione di questi quattro sistemi (nervoso, immune, cutaneo ed endocrino). Questa è la sfida futura di una società in continua evoluzione, dove la ricerca della salute e della bellezza sono richieste a gran voce da una popolazione che invecchia sempre di più ma vuole apparire giovane.

INTRODUCTION

Ageing may be regarded as a cellular process that, partly genetically determined, is influenced by endogenous and exogenous wear and tear. Characterised by a decrease in functional capacity and increased susceptibility to certain diseases and environmental insults it includes skin changes such as wrinkling, laxity, dryness, general thinning, and a flattening of the dermal/epidermal interface (1). Many of the skin collagen bundles appear, in fact, atrophic and elastic fibres are fewer and fragmented (Fig. 1). Thus, for formulating and producing anti-ageing cosmeceuticals and nutraceuticals capable of rejuvenating the skin, it should be necessary to know the intimate organisation of this important organ and, therefore, the global life-cycle of all its cells (2-4).

The skin

The skin is a multicellular organ comprising three distinct major compartments: the epidermis, dermis and hypodermis (Fig. 2) (5).
It has blood and nerve systems and contains many related appendages, some of which have an opening to its surface, such as pilosebaceous units and eccrine glands.

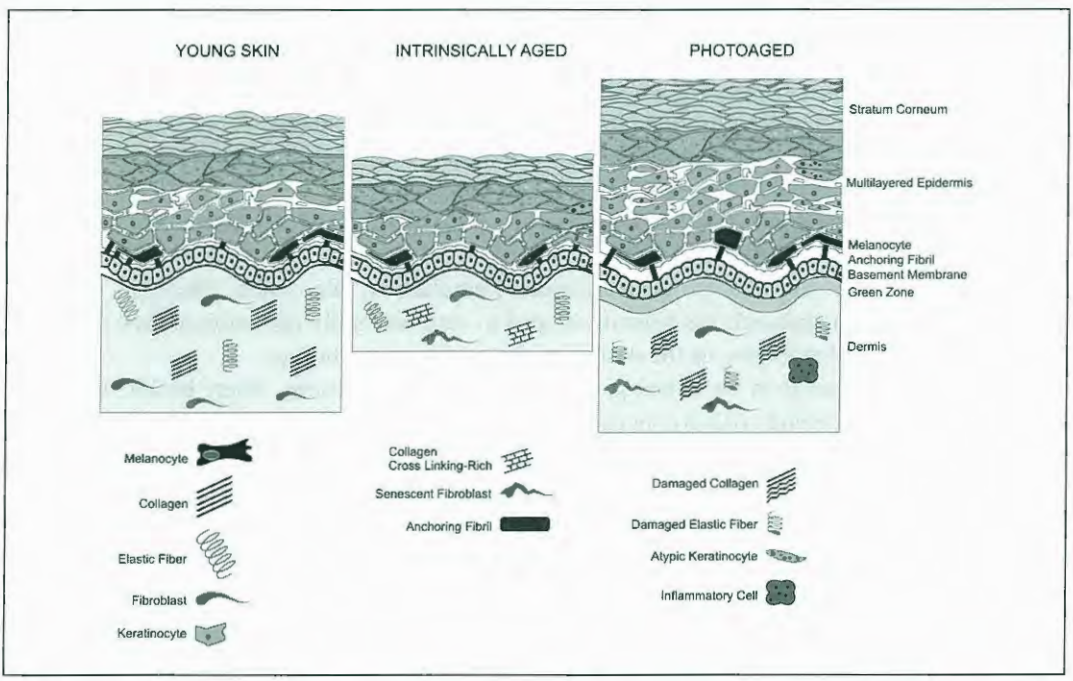

Fig. 1 *Young skin reveals a balanced composition and distribution of keratinocytes of the multilayered epidermis (E) and a normal stratum corneum (S), together with a normal anchoring of basement membrane (BM), and distinct extracellular matrix components, such as fibroblasts, elastic fibres and collagen fibres. The distribution of melanocytes is normal also. Intrinsically aged skin is atrophic with a decrease in epidermal and dermal thickness. The content of cross-links in collagen is increased, meanwhile both collagen and elastic fibres are reduced in quantity together with fibroblasts that become senescent. Photo aged skin presents hyperplasia with increase thickness of the stratum corneum (S), the epidermis (E) and the dermal compartment. The distribution of the melanocytes becomes inhomogeneous resulting in pigmentary changes, anchoring fibrils are reduced in number and microfibrillar components results severely damaged; long-term sun exposure also results in a state of inflammation.*

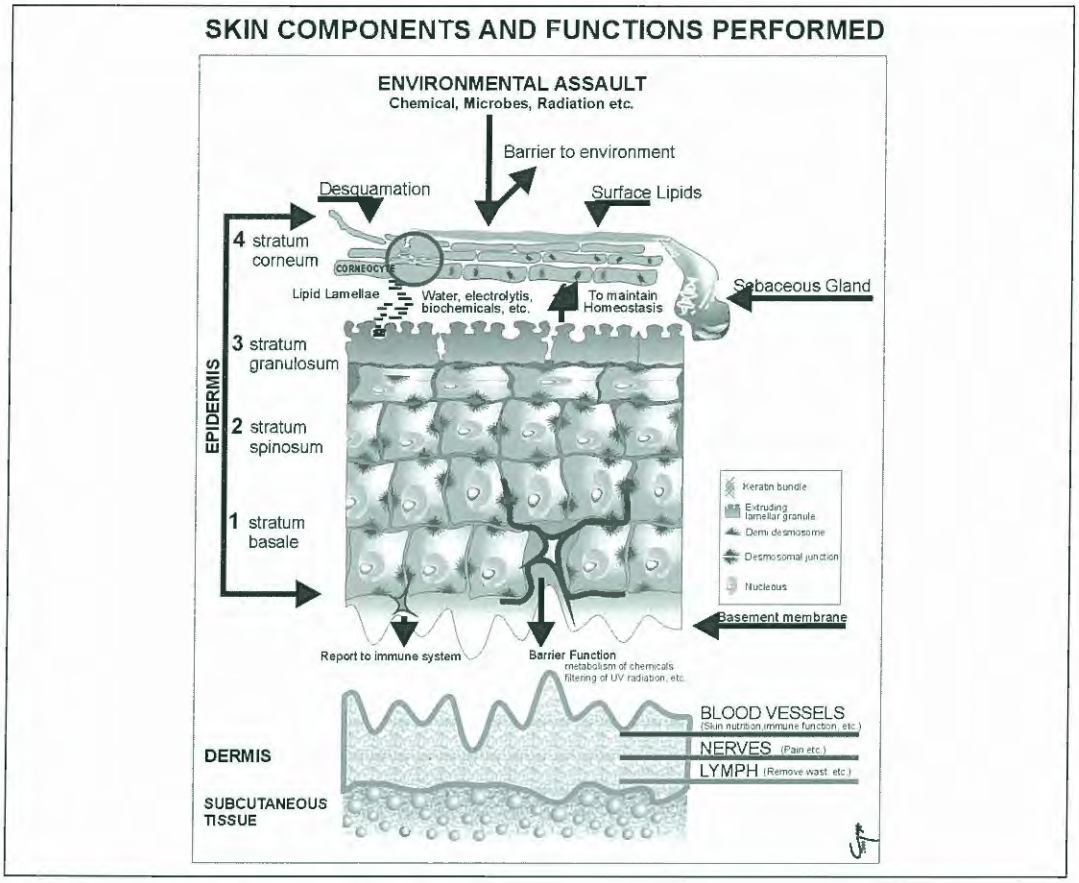

Fig. 2 Skin components and functions performed.

The epidermis contains three main cell types: the keratinocyte, the melanocyte and the Langerhans cell. Keratinocytes, continually migrating from the basement membrane (basal cells) differentiate themselves as outermost corneocytes which, embedded in lipid lamellae, give rise to the *stratum corneum* (SC). This dynamic structure, adapting to any environmental condition, represents the skin defensive barrier (6, 7). Another major characteristic concerning the epidermis is that its cells can produce the cytokine-messengers (signals), following stimulation. In this way it represents a key part of the body's early signal responses(8).

The dermis is a composite system of insoluble fibrils, collagen and elastin, embedded in soluble polymers (proteoglycans and hyaluronan) which, binding a vaste amounts of water helps to regulate cellular growth, adhesion, migration and differentiation.

The hypodermis (subcutaneous tissue), as adipose fatty layer, is composed mainly of trygliceri-des (fatty acids) and represents a potential energy store. Moreover, it provides insulation against the cold, maintaining body temperature and the enzymatic function. The exact composition of fatty acids reflects diet and environmental conditions.

The skin's homeostatic central mechanism is represented by a complex network of cytokines (peptidesmessengers) mediating interactions

between resident keratinocytes, Langerhans cells, T-lymphocytes, neutrophils and macrophages. Cytokines may be produced by many target cells and these may exert an effect on many cell types, binding to specific cell surface receptors. This peptide activates the surface receptor proteins which, acting as intracellular signals, alter the behaviour of the target cell.

Intercellular signals

Target cells use various intracellular mechanisms, including feedback loops, to adjust the ways in which they respond to extracellular signals. The positive feedback loops can help cells to respond in an all-or-none fashion to a gradually increasing concentration of extracellular signals or to convert a short-lasting signal into a longlasting one, or even to give irreversible responses. A delayed negative feedback allows cells to desensitise the signal molecules, enabling them to respond to small changes in the concentration of the signal molecule, over a large concentration range. Thus, for cells to function properly, they must organise themselves in space and interact mechanically with their environment. Moreover they have to be able to rearrange their internal components as they grow, divide, and adapt to changing circumstances.
This cell's communication network is, therefore, essential to regulate its own development through a bidirectional way of different signals, which means that cytokines can be regarded as the alphabet of the biologic regulatory language (Fig. 3) (8-10).

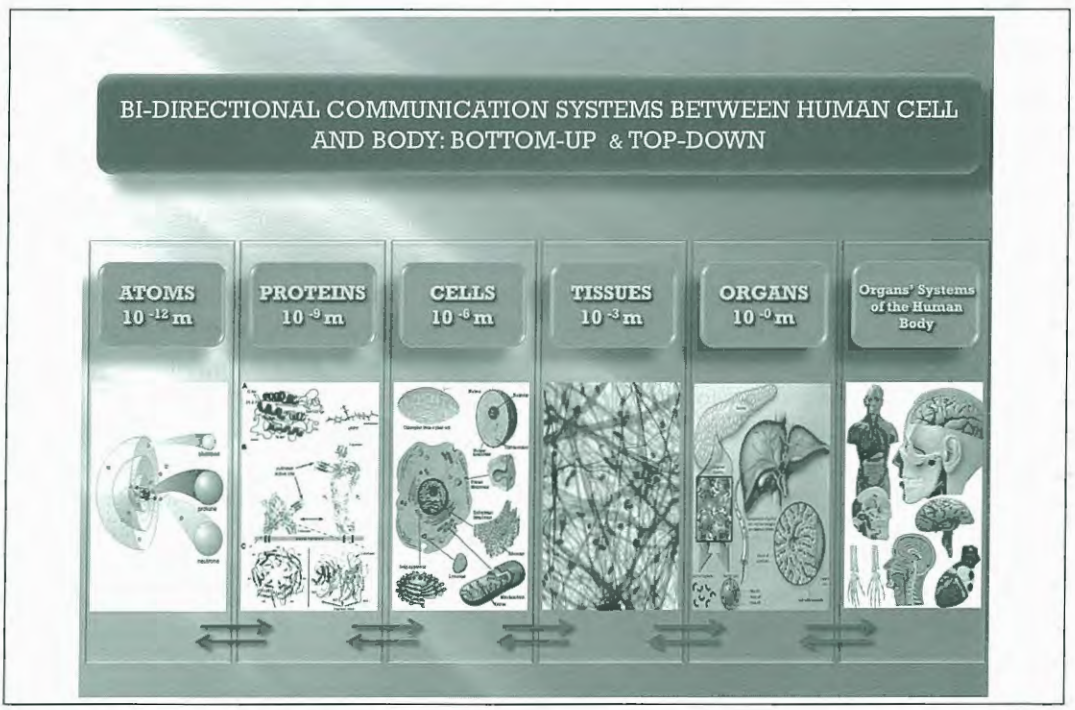

Fig. 3 Bi-directional communication systems between human cell and body: Bottom-up and top-down.

It has been shown that the cells may communicate in three different ways:

- By chemical compounds acting as signals for distance cells.
- By signal molecules associated with their own cell membrane, capable of inflnencing all the cells contacting.
- By gap junctions directly formed with the other cells' cytoplasm (Fig. 4).

However, the chemical signal expresses itself in the following three ways:

- The majority of cells secrete one or more chemical signals, acting as local chemical mediator.
- Specialised endocrine cells secrete hormones, travelling through the blood flux.
- Nervous cells act locally through their neurotransmitters.

Both endocrine and nervous cells are highly specialised units, capable of coordinating at long distance billions of other body cells, including those in skin. However, about 60/70 macromolecules, defined as hormones, neurotransmitters, growth factors etc., belong to a unique family of peptide messengers (11-13), and all these messengers link together accross the nervous, immune and endocrine systems.

These peptides represent the biochemical expression of the emotions and have a fundamental role to coordinate all the activity of the immune and endocrine systems, and also integrating all the mental, emotional and biological activities of the skin (Fig. 5).

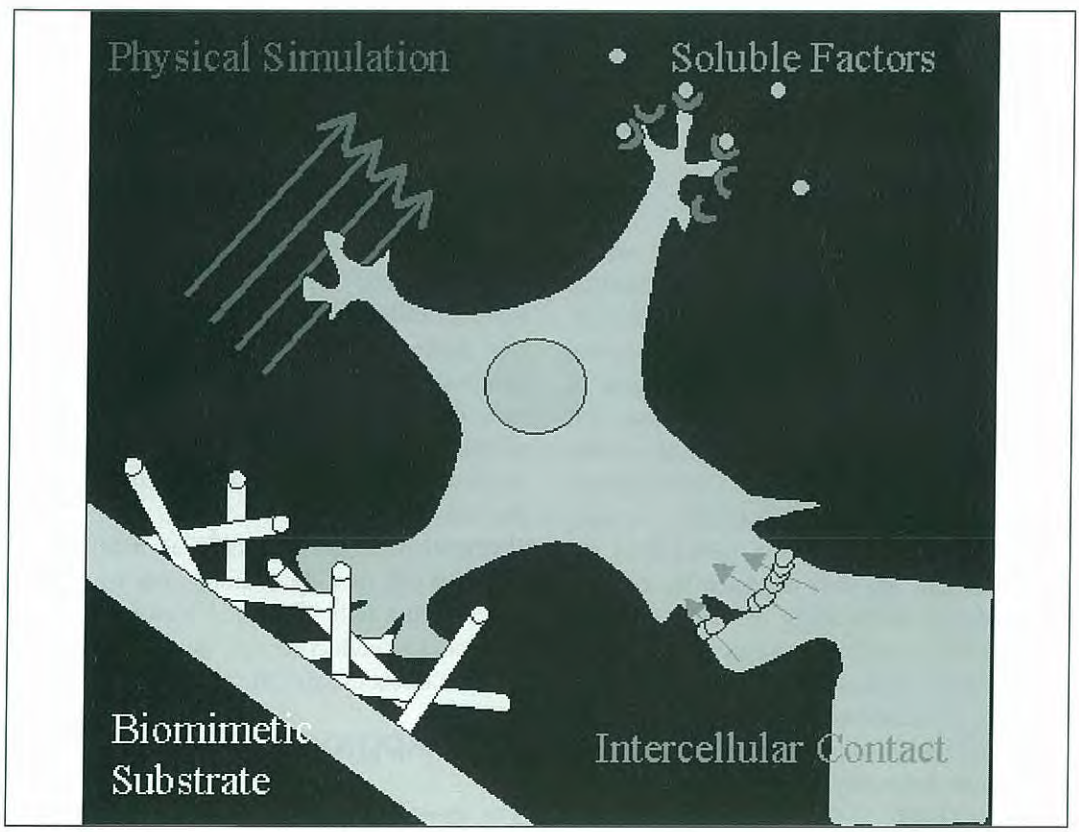

Fig. 4 *The signals as intercellular communication.*

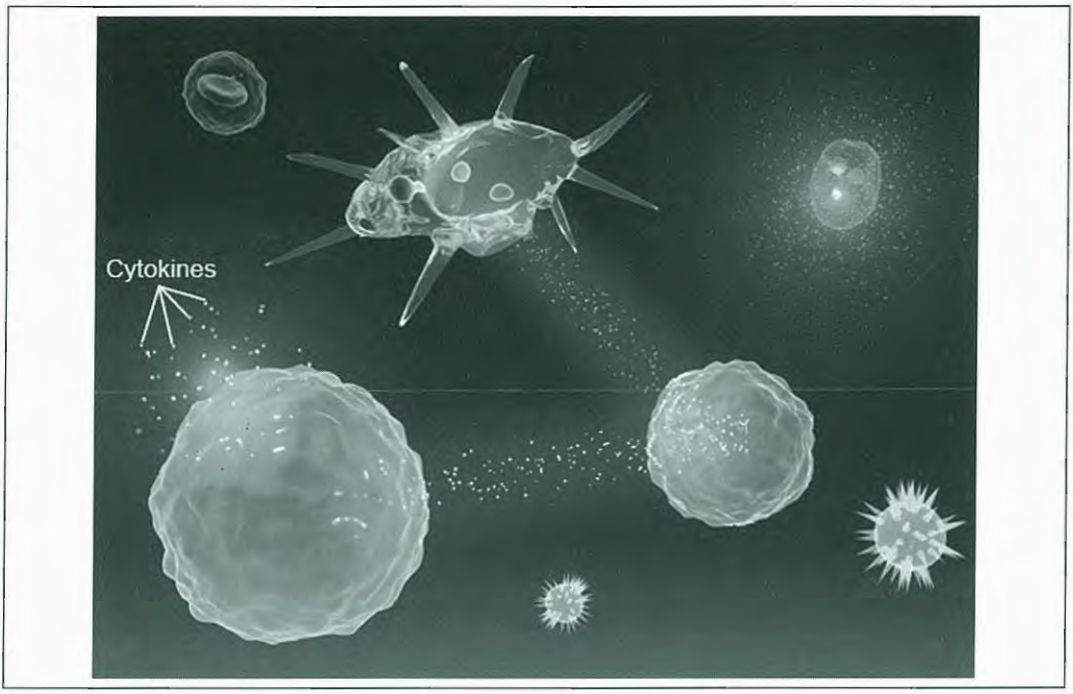

Fig. 5 The peptide-messengers.

NICE Approach

For all these reasons, at the level of "cosme-nutri-ceutical" formulations, the NICE approach has been developed to experimentally demonstrate how the nervous, immune, cutaneous and endocrine systems all work together to give us the desired global wellness and beauty (14, 15).
Apart from the well known anatomical connections, many other functional links exist connecting the skin with the nervous system. Vasodilatation, sebum secretion, perspiration, and erection of hair are thus regulated by different neuromediators activated by the nervous system and the skin's surveillance signals.
New studies have shown that the immune system is directly connected with the endocrine system, which send signals to the nervous system and the skin system, defending it from the environmental insults (16-19).
The majority of chemical signals (peptides) influence in different ways any target cell, modifying protein synthesis, or producing new, proteins in accordance with the specific replies from different protein receptors. These signals may be represented by hydro or lipo-soluble molecules. The hydrosoluble molecules may struggle to penetrate through the cellular lipidic membrane, only linking themselves to specific superficial protein receptors (18, 19) while the liposoluble molecules penetrate quickly, directly acting in the cell cytosol.
Furthermore progression from an indifferentiated stem cell that, for example, has no specific function to a fully functional nervous, immune, cutaneous or endocrine cell, involves a complex cocktail of chemicals (20, 21).

TMC Approach

The theory of ying-yang used to explain the cause of diseases of human body, was the most

significant concept that reflected the phylosophic thinking of people in ancient China.
The sages in ancients believed that the heaven and the earth have formed a big universe while the human body itself has constructed a small universe. The big universe and the small universe communicate and interact with each other.
According to the idea of cultivating health in the four seasons, all things begin to sprout in Spring, grow in Summer, ripen in Autumn, and store up in Winter.
Such an analogy was used quite often in the medical books completed in different dynasties in Chinese history especially in establishing the theory and developing the therapeutic methods of TCM.
Interaction between the heavens and human beings is therefore, an important part in the framework of Chinese people's thinking, as the interactions between our skin cells and the environment has been recognized fundamental from Western medicine for the wellbeing of our body.
In TCM cutaneous, endocrine, immune, and nervous systems though possessing their own characteristics, are interrelated to each other also. Thus similarity between the NICE and the TMC appoach to formulate innovative neurocosmetics.

Cell organisation

The cell is organised as a closed feedback loop responding to internal and external environment (19-22). Each cell has a membrane containing a cellular fluid, organised as a molecular soup and composed of nutritive chemicals, necessary to make all the other components (23, 24).
Suspended in the intracellular fluid there are: the cell nucleus, which is represented by a high number of little factory-centres where the basic components of the cell structures are made; it also contains many other specialised centres (organelles) (25). The most important organelles are necessary to store raw materials, recycle metabolites, and/or produce energy to support all the cell's necessities. In a similar way to that of a single cell, these organelles are all surrounded by membranes selecting, from its cytoplasm, each ingredient that is coming in or going out (26-28). In particular, the cell membrane accepts the incoming of the nutritive ingredients, eliminating the rejected items (29).
The cell nucleus contains DNA molecules with genetic information, and RNA messengers, made from DNA, to give information to the production centres (30). Thus, the genetic information is transferred from DNA to the system capable of producing the cell proteins by the activity of the RNA messenger. In every gene is written the instruction to make a protein.
The nuclear genome ("genome" represents the total genetic content of the set of chromososmes in a nucleus or in an organelle) is subdivided into chromosomes, the molecular vehicles, enabling the sets of genes to be duplicated.
The backbone of each chromosome is a linear piece of DNA, containing thousands of genes from 30 to 60,000 genes! Thirty-thousand are in a continuous activity, and they have the capacity to regulate the sole activity and functionality of the intelligent protein molecules. Other 30,000 genes, named AVATAR are only of necessary support as controller.
These genes regulate and control the regular transcription of the informations. Within the nucleus there is a mininucleus, the nucleolus, a ribosome producing factory, processing and assembling RNAs, RNA-processing enzymes, ribosomal proteins, etc. The factory-centres (organelles) of granular compositions are complex catalytic machines, the ribosomes, made from more than 50 different proteins and several RNA molecules, in which protein synthesis is performed. These comprise the structural proteins and the enzymes necessary to promote all the cellular processes.

In every cell there are about 5,000 factoryproducing centres (31).

Other flattened membrane-enclosed compartments, called cisternae (Golgi apparatus), are storage bags resembling a stack of pita breads. Into these bags are stored different cellular products which are classified, stocked, and mailed to the final destination. The recycling centres (lysosomes) are organelles containing enzymes capable of digesting nutritive ingredients, damaged components of the cell, and many molecules no longer used. The catabolised single elements are then recycled to make new cellular components (32, 34).

The electrical locations (mitochondria and peroxisomes) are necessary for the cellular breathing. The cells use oxygen to reduce the organic molecules in CO2 and H_20. The released energy, coming out during this process, is fixed on special molecular energy-carriers to be used for all the other cellular processes (cellular metabolism). These electrical locations have their own genetic material and may produce independently from the cell reproductive processes.

In summary, into every cell it is possible to distinguish (Fig. 6) the cytoplasm, or cellular fluid; the nucleus and nucleolus, ribosome-producing factories, where RNAs are produced and other RNA-protein complexes are assembled. The endoplasmatic reticulum (ER), a membrane organelle in which proteins and carbohydrates are processed, is a structure organised into a net-like labyrinth of branching turbules and flattened sacks that extends throughout the cytosol. The Golgi apparatus, special storage-bags where sugar nncleotides and glycoproteins (proteoglycans) are processed and generated, is also a dispatching station for products of the ER. The mitochondrion that is central energy source contains mitochondrial DNA and ribosomes, and houses protein synthesis generating ATP (Adenosinetriphosphate) from the oxidation of food molecules. The peroxisomes, sites of oxygen utilisation, perform the oxidative functions not taken by mitochondria. The lysosomes are membrane-enclosed compartments filled with soluble hydrolytic enzymes that recycle many ingredients, controlling digestion of macromolecules.

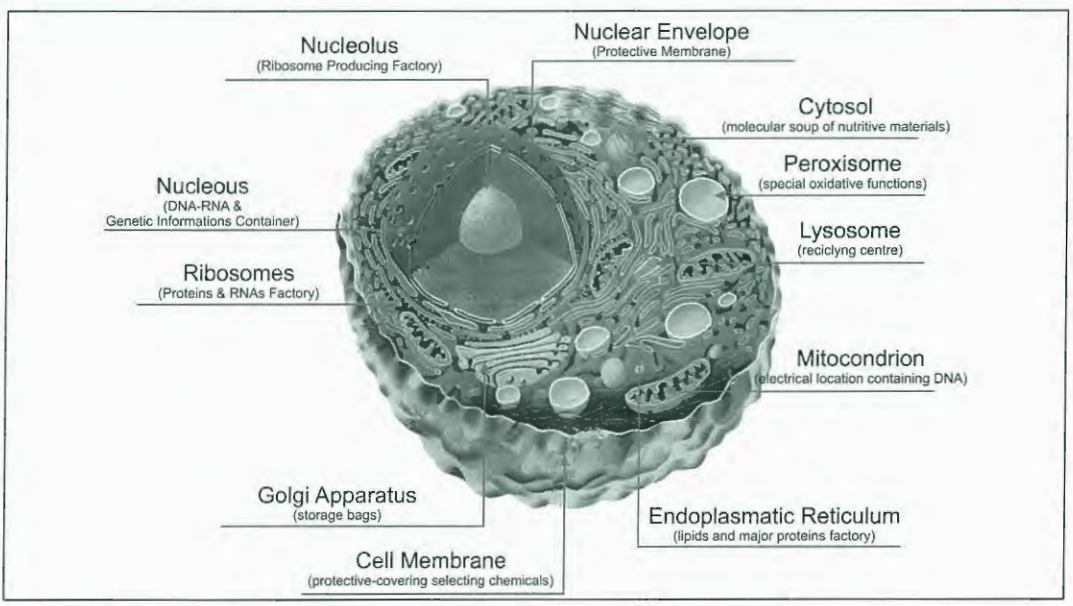

Fig. 6 The cell autopoietic activity.

They represent meeting places where several streams of intracellular traffic converge (35, 36). The cellular global pattern is, however, very complex because of all the cellular components interconnected with each other by a feedback loop in a net involving thousands of processes.
Thus, a common aim in skin biology is to understand the function of genes within a particular pathway (developmental and metabolic), their contribution to how skin and the connected tissues /organs work, and what it is that makes one skin or tissue cell different from another.
Coming back to the NICE concept, all the body cells and therefore the cells of the skin, are connected with the brain structure. The human brain is estimated to contain 50-100 billion nerve cells or neurons, and these cells pass signals to each other via synaptic connections. One reason the brain functions in such a subtle manner lies in the way messages pass from neuron to neuron. Chemical signals (neurotransmitters) travel, from one nerve cell to another across a gap or synapse. Once the molecule locks into the recipient neuron, the chemical can decay, having done its job, and the electrical message continues on its way. The synapses number as many as 1000 trillion! (Fig. 7)

As we have seen elsewhere, there are many other molecular players involved in the development of the skin cells, for example the immune and endocrine systems. In this way the intercellular complexity increases exponentially if we think tbe modality by which all the cellular components are interconnected with each other in a very large unique net, involving thousands of different processes (37-39).
To understand the cellular and intercellular networks, we try to imagine a single net.

Autopoietic organisation

DNA of the cell nucleus produces molecules of RNA, which in turn contain the information necessary to produce proteins, enzymes included. Some of these enzymes are specialised to select, take off, and replace damaged elements of DNA. On the other hand, DNA produces RNA, which gives the instructions to the producing-factories to generate the enzymes necessary for repairing DNA (Fig. 8). Thus, each component of this net contributes to produce or transform other cellular components by a mechanism of autopoiesis (40-42).

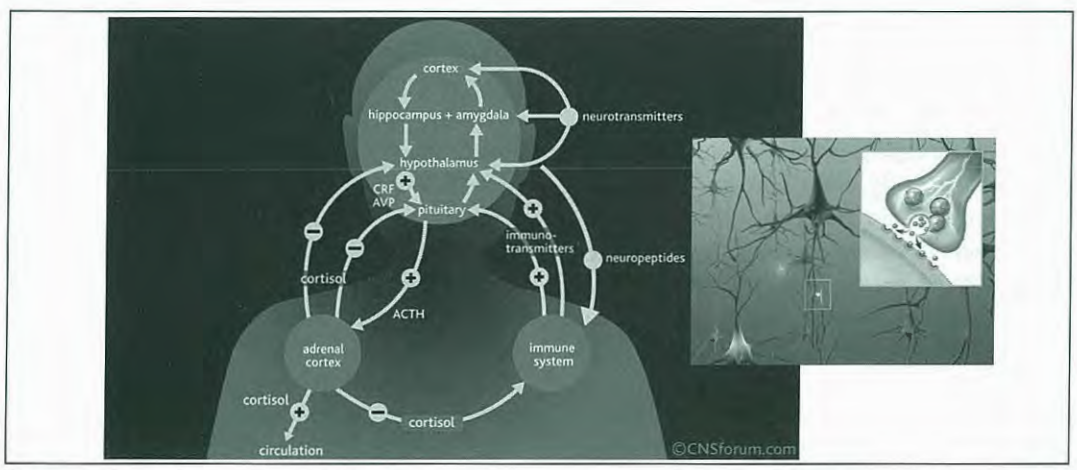

Fig. 7 The electrical messages through the synapses.

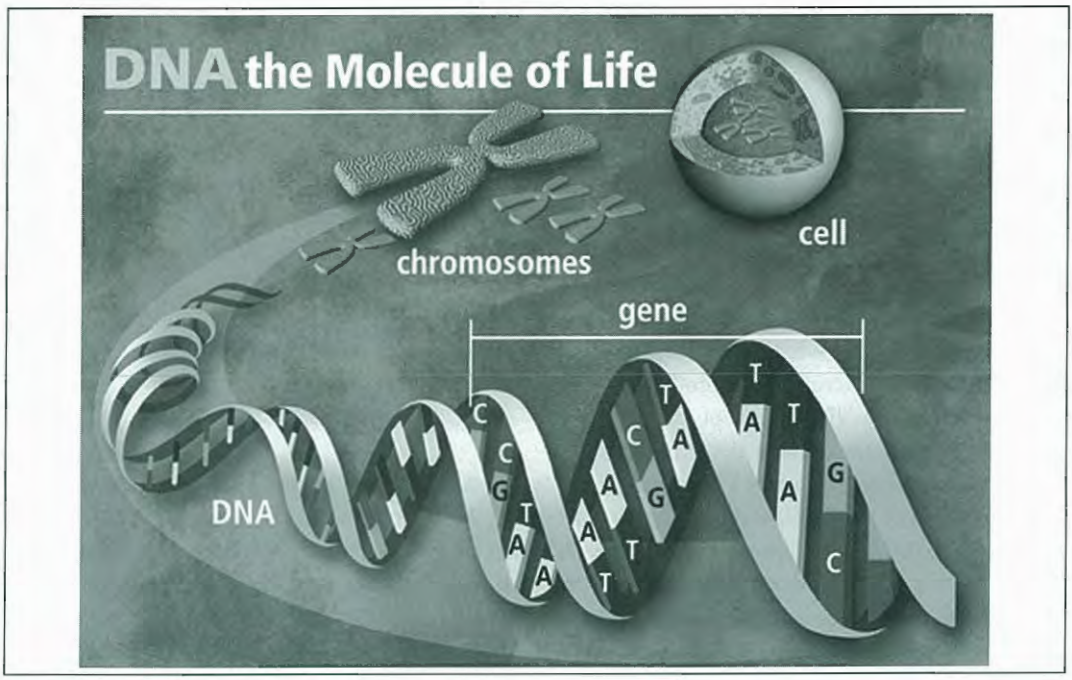

Fig. 8 DNA –the molecule of life.

To complete this picture it is necessary to add other cellular basic elements necessary to produce DNA, RNA, and the relative enzymes that are the centrals of energy, coming from the sugars breakdown, indispensable to all the productive processes.

In conclusion, each component of the cell net contributes to produce and transform other components of the same network by an autopoietic mechanism.

In any way, autopoiesis is the living mechanism of the human body and all of its components, comprising the skin and of course, all the cells, which reacts to environment aggressions continuously modifying its own structure under the influence of the nervous, immune, cutaneous and endocrine system (NICE). Therefore, the skin interchanged energy and the ingredients taken from food and from the cosmetic products, must help to modulate cell organisation and rebalance its global structure. Of course, all the cosmetic ingredients should not negatively interfere with the skin components, but help their metabolic productive processes. Multifunctional cosmetic ingredients have to create a seemingly infinite number of possibilities for development pathways in skin systems. Given the complex nature of humans, understanding all the skin structure activities is often perplexing. The key to interpreting the skin's activities lies within the real knowledge of neuroscience, immunology, endocrinology, and more specifically within what is known about the skin and cosmetic science.

In an effort to evaluate the role it plays in connection with other body networks, such as the nervous, immune, and endocrine systems, the skin organisation at the anatomical, physiological and functional level, is of great importance to understanding its fundamental functions.

Similarly, the deep knowledge of the cosmetic ingredients' mechanism of action, together with

the activity of the used vehicles to trap and deliver them through the skin layers, are of fundamental importance to understanding how they may interact with the everyday cell life.

How to correct or control the cell dynamic is, therefore, essential to cosmetic efficacy and safety (43-46).

Thus, for example, specific studies should help take the potential of genomics to identify the regulators controlling specific genes. Genomics is the study of the workings of the complex web of genes and the factors that regulate them. Each cell has just one genome, but with that one genome it can cope with multiple challenges with other cells. Genes are constantly being switched on and off, and the molecules that switch genes are called genetic regulators or transcription factors.

These transcription factors bind to particulate sites on a chromosome to turn nearby genes on or off. The consequent pattern of gene activity is, thus, responsible for the cell development, function, and response to environmental challenges and stimuli. Malfunction or good performance in this system can cause disease or wellness (47-49).

For all these reasons it is necessary to better understand the mechanisms governing the cell life and the skin as an organ.

Genome information

The cell types in a multicellular organism, as in humans, become different from one another because they synthesise and accumulate different sets of RNA and protein molecules.

Their DNA has a central role in defining the sequential programme of development, calling genes into action at specific times and places, according to the pattern of gene expression present in each cell at the previous development stage (50-52).

Sequences of simple signals, acting at different tissues and places in growing cell arrays, give rise to the intricate and varied multicellular organisms. The genome of each cell contains in its DNA sequence the information to make many thousands of different protein and RNA molecules, so that its sequencing provides an understanding of the genes' necessities (53). A cell typically expresses only a fraction of its genes, and the different types of cell, in multicellular organisms arise because different sets of genes are expressed (Fig. 9).

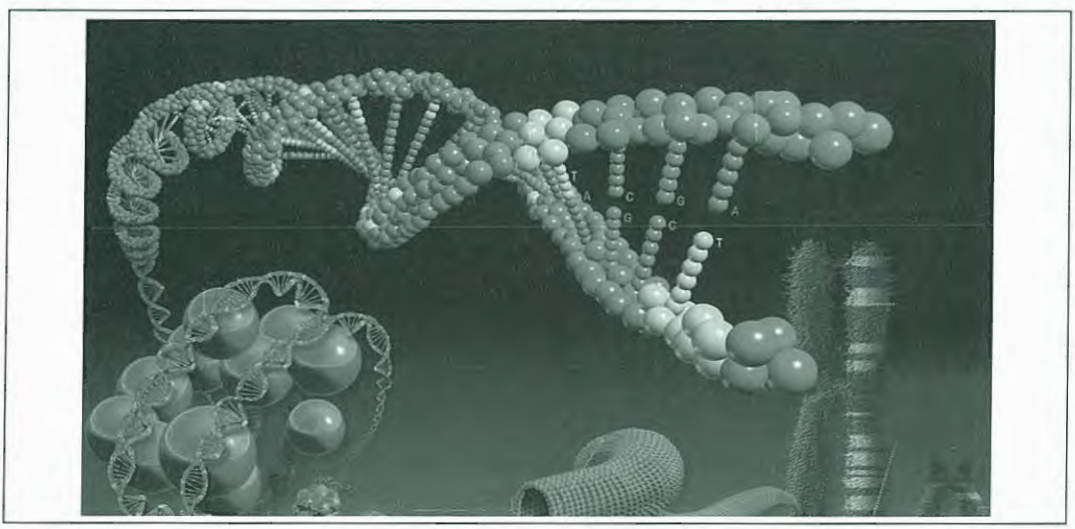

Fig. 9 *The human genome.*

Moreover, cells can change the pattern of genes they express in response to changes in their environment, such as signals from other cells. In any way many processes are common to all the cells that have many proteins in common. These include the structural proteins of chromosomes, RNA, polymerases, DNA repair enzymes, ribosomal proteins, enzymes involved in the central reactions of metabolism, and many of the proteins that form the cytoskeleton (54).

The organism's DNA encodes all of the RNA and protein molecules required to construct its cells, so that all the cell types become different from one another. They synthesise and accumulate different sets of RNA and protein molecules, thus becoming dramatically different in both structure and function. Therefore, this cell differentiation generally depends on changes in gene expression rather than on any changes in the nucleotide sequence of the cell's genome, according with our knowledge today. This is the reason why progress in science is often driven by advances in technology.

Living cells deal information

The complexity of humans depends, however, on a remarkable feature of the genetic control system. Cells memorise the genes a cell expresses, and the way it behaves depends on the cell's past as well as the present environment. All the different cells maintain their specialised characters not because they continually receive the same instructions from the surrounding, but because they retain a record of signals their ancestors received in early embryonic development (55-57).

Living cells, like computers, deal in information, storing their hereditary information in the form of double-stranded molecules of DNA-long unbranched paired polymer chains, formed of four types of monomers. These monomers are strung together in a long linear sequence that encodes the genetic information, just as in a computer file.

To carry out its information-bearing function, DNA must do more than copy itself. It must also express its information, by letting it carry out the synthesis of other molecules in the cell. This occurs by a mechanism leading first and foremost to the production of two other key classes of polymers: RNAs and proteins. The process begins with a template polymerisation called transcription, in which segments of DNA sequences are used as templates for the synthesis of shorter molecules of the closely-related polymer ribonucleic acid, RNA. Later, in the more complex process of translation, many of these RNA molecules direct the synthesis of polymers of a radically different chemical class; the proteins.

CONCLUSION

In conclusion, there are many biochemical processes that occur outside and inside the cells. Membranes bind the enzymes, which catabolise the metabolic processes of lipids and the oxidative phosphorylation process, necessary for ATP synthesis at the level of mitochondria. The endoplasmatic reticulum (RE) is the producer of almost all cell lipids, as well as of many proteins synthesised into its own cytosol.

The synthesis and catabolism of signal molecules are regulated by many enzymes capable of synthesising some lipids and/or amino acids.

However, all these processes are organised and enclosed in the genome of DNA (58). Thus, the amino acid sequence dictates, for example, how each protein folds to give a specific molecule. But when a cell makes a particular protein, it must decode accurately the corresponding region of the genome. Moreover, additional information encoded in DNA of the genome specifies exactly when and in which cell type each gene is to be expressed into protein. And since

proteins are the main constituents of cells, the decoding of the genome determines not only the size, shape, biochemical properties, and behaviour of cells, but also the distributive features of every species on Earth.

Coming back to the skin, the epidermis is a self-renewing tissue in which a loss of cells from the SC is balanced by cell growth in the Stratum basale (deeper layer before dermis), governed by continuous cell-signals, energy consumption and nutrients supported by the dermis.

The innovative nutricosmetics of the last generation have to give the skin all the elements necessary for its survival, but they also have to be capable of ameliorating its state of health, decreasing skin ageing as much more possible. Through the new era of the NICE-TCM approach will be possible to achieve this ambitious goal.

Skin life and energy management is a delicate balance between intake rate of production and use and storage in the cell of fatty acids, amino acids, polypeptides and proteins, sugar and polyglucosides, and water. Many aspects of skin life have been discovered, while the fundamental mechanism of the feedback loop of cells remains deeply mysterious. We are able to control the growth of different skin cells in culture, but still now we cannot see what is happening in the skin at cellular level.

Thus, we are a long way from being able to mimic the complexity of the signals, and feedback messages interchanged among the skin and other cells of our body. In conclusion, we do not know the mechanisms regenerating the systemic biological management and, thus, the cell autopoiesis, at the base of the body and skin homeostasis.

However, we know that the autopoietic mechanism regulating the body's skin cells is due to a sum of continuous interrelations between different anabolic and catabolic processes, so that cells and tissues are always in a state of dynamic equilibrium. When these processes stop, cell stop to live. In our body there is, in fact, a large family of peptides which, acting as molecular messengers and signals, establish a unique net of communication among all the cells.

In this way every cosmetic product applied to the skin should act not only at the level of the lipid lamellae and corneocytes, but should also have the capacity of generating a global wellness, involving the complex chemical net of peptides all around the body (14, 15, 22).

In summary, the most important factor in developing an innovative cosmetic product is recognising that innovation is not an isolated activity. It is both the result and driver of knowledge on skin science between cosmetic chemists, and all the more important, scientists of the biological and medical community.

Every day our society becomes older, and seeks to rejuvenate in a short time looking forever youthful. So, the focus should be on the production of innovative cosmetic products caractherized for a higher quality, efficacy, and safety for human body and the environment.

Thus, the major trend, influencing the future personal care market, will be to obtain health and beauty from outside and inside by the contemporary use of cosmeceuticals and nutraceuticals based on the new NICE/TMC approach (59-62). But for obtaining these innovative products it will be necessary to consider the complexity of the skin connected with the music of the human genes, the harmony of the body cells, and the symphony of their organisation. Therefore, by the union of selected active ingredients and a well balanced carrier and an adequate study project to control the efficacy and safety of the final formulation product, it will be possible to realise a harmonic musical composition (the cosmetic product) capable of playing in unison with the symphonic organisation of the human cells, producing emotions, beauty and wellness.

In conclusion, the strategy of all cosmetic manu-

facturing companies should be to promote their own innovation by an appropriate management (Fig. 10) and the free movement of their research professionals, together with a significant increase in R&D spending. This is the future challenge in the actual global and transforming society, where health and beauty concern become the ongoing quest for an ageing population.

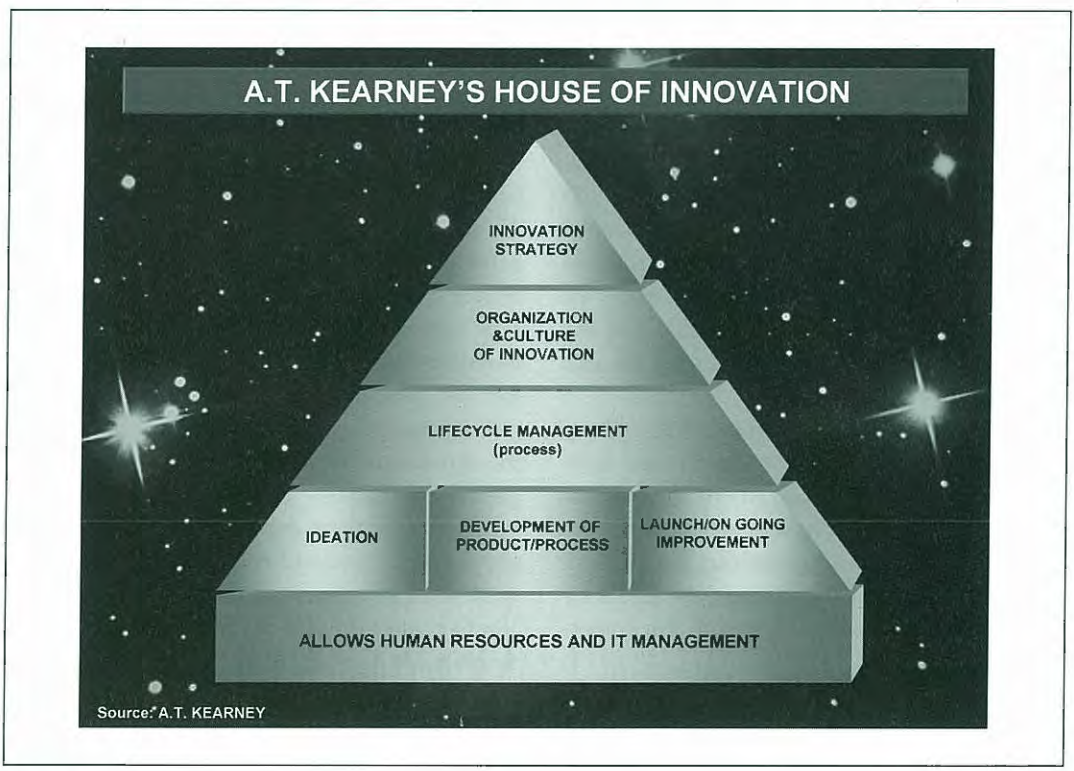

Fig. 10 Management of innovation.

References

1) **Gilchrest BA, Krutmann J. (2006)** *Skin aging*. Berlin Springer Ed.
2) **Laszlo E. 1987)** *Evolution*. Boston Shambhala Ed.
3) **Lyun M. (1993)** *Symayosin in cell evolution*, 2nd Ed., San Francisco, Freeman Ed.
4) **Alberts B, Johnson A, Lewis L, Raffe M, Roberts K. (2008)** *Molecular biology of cell*, 5th Ed. New York, Garland Science Ed.
5) **Priestley GC. (1993)** An introduction to the skin and its diseases. In: GC Priestley Ed Molecular aspect of Dermatology, New York, John Wiley & Sons; pp.1-17.
6) **Elias PM. (1983)** Epidermal lipids, barrier function, and desquamation. *J. Invest. Dermatol.*, **80 (Suppl):** 445-95.
7) **Rawlings AV, Scott IR, Harding CR, Bowser PA.(1994)** *Stratum corneum* moisturization at the molecular level. J, *Invest Dermatol.*, **103 (5):** 731-41.
8) **Corrk MJ, Mee JB and Duff GW. (1993)** *Cytokines* In: Priestley Ed Molecular aspect of Dermatology, New York, John Wiley & Sons; pp.129-46.
9) **Berridge MJ. (2005)** Unlocking the secrets of cell signalling. *Ann. Rev. Physiol.*, **67:** 1-21.
10) **Breer H. (2003)** Sense of smell: recognition and transduction of olfactory signals. *Biochem. Soc. Trans.*, **31:** 113-16.
11) **Pert CB, Ruff MR, Weber RJ, Herkenham M. (1985)** Neuropeptides and their receptors: a psychosomatic network. *J. Immunol.*, **135 (2 Suppl):** 820-26.
12) **Pert CB. (1992)** Peptide T: *a new therapy for AIDS*. Symposium, N° V5, S. Francisco, USA Unpublished. Disposable on cassette. Advances Peptides Inc, 25 East Loup Road, Story Book, NY.
13) **Pert CB. (1993)** *The chemical communication interview.* In: Bill Moyers, Healing and the Mind, Doubleday, New York.
14) **Morganti P, Palombo M, Palombo P, Fabrizi G, Cardillo A, Carezzi F, Morganti G, Ruocco E, Dzierzgowski S. (2010)** Cosmetic Science in Skin Aging: Achieving the Efficacy by the Chitin Nano- Strucured Chrystallites. *SOFW Journal*, **136 (3):** 14-24.
15) **Morganti P, Yuan-Hong Li, Hong-Duo Chen. (2011)** NICE melody for innovative mind-body skin care. In print on: Cosmetic Science Technology.
16) **Torii H, Yan Z, Hosoi J, Granstein RD. (1997)** Expression of neutrophic factors and neuropeptide receptors by Langerhans cells and the Langerhans cell-like line XS52: further support for a functional relationship between Langerhans cels and epidermal nerves. *J. Invest. Dermatol.*, **109:** 586-91.
17) **O'Sullivan RL, Lipper G, Lerner EA. (1998)** The Neuro- Immuno-Cutaneous-Endocrine Network: Relationship of mind and skin. *Arch Dermatol.*, **134:** 1431-5.
18) **Querleux B, Danchot K, Jourdain R et al. (2008)** Neural basis of sensitive skin: an fRMI study. *Skin. Res. Technol.*, **1:** 1-8.
19) **Hosoi J. (2009)** Establishment and change of NICE approach. *J. Appl. Cosmetol.*, **27:** 200.
20) **Deisseroth K, Mermelstein PG, Xia H, Tsien RW. (2003)** Signalling form synapse to nucleus: the logic behind the mechanisms. *Curr. Opin. Neurobiol.*, **13:** 354-65.
21) **Luttrell LM. (2006)** Transmembrane signalling by G protein-coupled receptors. *Methods Mal. Biol.*, **332:** 3-40.

22) **Morganti P. (2010)** The cosmetic activity at cell level. *Eurocosmetics.*, **18 (1, 2):** 24-26.
23) **Hosoi J. (2011)** NICE-network. Evidence and application to cosmetics. *J Appl Cosmetol.* **29:** 75-86
24) **Maturana H, Varela F. (1987)** *The tree of knowledge.* Boston, Shambhala Ed.
25) **Blobel G. (1980)** Intracellular protein topogenesis. *Proc. Natl. Acad. Sci. USA*, **77 (3):** 1496-500.
26) **Koehler CM, Merchant S, Schatz G. (1999)** How membrane proteins travel across the mitochondrial intermembrane space. *Trends Biochem Sci.*, **24 (11):** 428-32.
27) **Truscott KN, Bradner K, Pfanner N. (2003)** Mechanism of protein import into mitochondria. *Curr Biol.*, **13 (8):** R326-27.
28) **Mokranjac D, Neupert W. (2005)** Protein import into mitochondria. *Biochem. Soc. Trans.*, **33 (5):** 1019-23.
29) **Prakash S, Matouschek A. (2004)** Protein unfolding in the cell. *Trends Biochem Sci.*, **29 (11):** 593-600.
30) **Hastwell L, Hood L, Goldberg ML et al. (2006)** *Genetics from genes to genomes.* 3rd ed, Boston, McGraw Hill.
31) **Feuk L, Carson AR, Scherer SW. (2006)** Structural variation in the human genome. *Nat. Rev. Genet.*, **7 (2):** 85-97.
32) **Burgess TL, Kelly RB. (1987)** Constitutive and regulated secretion of proteins. *Annu. Rev. Cell. Biol.*, **3:** 243-293.
33) **Anderson RG. (1998)** The caveolae membrane system. *Annu. Rev. Biochem.*, **67:** 199-223.
34) **Eligoard L, Helemins A. (2003)** Quality control in the endoplasmatic reticulum. *Nature. Rev. Moll. Cell. Biol.*, **4:** 181-191.
35) **Conner SD, Schmid SL. (2003)** Regulated portals of entry into the cell. *Nature*, **422 (6927):** 37-44.
36) **Bonifacino JS, Traub LM. (2003)** Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu. Rev. Biochem.*, **72:** 395-447.
37) **Foerster von It, Zopf GF. (1962)** *Principles of selforganization.* New York Pergamon Press.
38) **Margulis L. (1993)** *Symbiosis in cell evolution* 2nd ed. San Francisco, Freeman ed.
39) **Bateson P. (2004)** The active role of behaviour in evolution. *Biology and Philosophy*, **9:** 283-298.
40) **Maturana HR, Varela FJ. (1973)** *Autopoiesis: the organization of the living.* Santiago del Cile, Editional Universitaris.
41) **Smith M. (1994)** Nobel lecture. Synthetic DNA and biology. *Biosci Rep.*, **14 (2):** 51-66.
42) **Melloc C, Conte D. (2004)** Revealing the world of RNA interference. *Nature*, **431 (7006):** 338-42.
43) **Maynard SJ, Szathmary F. (1999)** *The origins of life: from the birth of life to the origin of language.* New York Oxford University Press.
44) **Stelling J, Klamt S, Bettenbrock K, Schuster S, Gilles ED. (2002)** Metabolic network structure determines key aspects of functionality and regulation. *Nature*, **420 (6912):** 190-3.
45) **45 Morganti P, Morganti G, Fabrizi G, Cardillo A. (2008)** A New Sun to Rejuvenate the Skin. *J. Appl. Cosmetol.*, **26:** 159-166.

46) **46 Morganti P, Hong-Duo Chen, Gao XH, Li Y, Jacobson C, Arct J, Fabianowsky W. (2009)** Nano science: the challenging cosmetic healthy food & biotextiles, *SOFW Journal,* **135 (4):** 2-7.

47) **Vender JC, Adams MA, Mayer EW et al. (2000)** The sequence of the human genome. *Science,* **291:** 1304-1351.

48) **Lockhart DJ, Winzeler EA. (2000)** Genomics, gene expression and DNA arrays. *Nature,* **405 (6788):** 827-36.

49) **Boone C, Bussey H, Andrews BJ. (2007)** Exploring genetic interactions and networks with yeast. *Nat. Rev. Genet.,* **8 (6):** 437-449.

50) **Ausubel FM, Brent R, Kingston RE et al. (2002)** *Short protocols in molecular biology.* 5th ed. New York, Wiley.

51) **Brown TA. (2002)** *Genomes 2.* 2nd ed, New York, Wiley- Liss.

52) **Watson JD, Caudy AA, Meyers RN, Witkowski JA. (2007)** *Recombinant DNA: genes and genomes.* A short course 3rd Ed, New York, WH Freeman.

53) 53 International Rice genome sequencing project. The map-based sequence of the rice genome. *Nature,* **436 (7052):** 793-800.

54) **Lewin B. (2008)** *Genes IX.* Boston, Jones & Bartlett Publish.

55) **Coen E. (1999)** *The art of genes: how organisms make themselves.* Oxford, Oxford University Press.

56) Novartis Foundation. *Complexity in Biology Information Processing.* Chichester, Wiley ed.

57) **Colvis CM, Pollock JD, Goodman RH, Impey S, Dunn J, Mandel G et al. (2005)** Epigenetic mechanisms and gene networks in the nervous system. *J. Neurosci.,* **25 (45):** 10379-89.

58) **Knight ZA, Shokat KM. (2007)** Chemical genetics: where genetics and pharmacology meet. *Cell.,* **128 (3):** 425-430.

59) **Morganti P. (2009)** Chitin nanofibrils in skin treatment. *J. App.l Cosmetol.,* **27:** 251-70.

60) **Morganti P. (2010)** *Chitin nanofibrils and their derivatives as cosmeceutical.* In: Se-Kwon Kim Ed. Chitin, Chitosan, oligosaccharides and their derivatives, New York, CRC Press, pp. 531-42.

61) **Morganti P, Li Y, Morganti G. (2008)** Nanostructured products: technology and future. *J. Plastic. Dermatol.,* **4 (3):** 253-60.

62) **Morganti P. (2009)** *Beauty from the inside and the outside. Natural products work in multiple ways.* In: (Tabor A and Blair R Eds) Nutritional Cosmetics. Beauty from within. William Andrew, Elsevier Group pp95-111.

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NICE network. Evidence and application to cosmetics

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Key words: *Stress; Hormon; Neuropeptide; Barrier; Langerhans cell;*

Summary

It is well known that mental stress affects the body and that the skin reflects a person's mental condition. Thus, proverbs, such as "The skin is the mirror which reflects the state of the mind", "The skin is a window to the mind." have developed and scientific evidence supporting this relationship has been accumulated. Examples of the effects of stress, their mechanisms, and modulation by cosmetics are reviewed in this article.

Riassunto

È ben noto come lo stress mentale influenzi il benessere del nostro corpo, mentre la pelle ne riflette le conseguenze.
"La pelle è lo specchio che riflette lo stato della mente" e che la pelle sia lo specchio della mente è ormai supportata da molte evidenze scientifiche.
Questo articolo riporterà alcuni esempi a dimostrazione degli effetti provocati dallo stress, dei loro meccanismi di azione e del come i prodotti cosmetici ne possano modulare l'attività.

INTRODUCTION

Effects of stress

Although the skin is located at the outer most periphery of the body and appears simple, it performs various functions that protect the whole body, including providing a barrier of water/bacteria/chemicals, shading from UV radiation, and removal of invading bacteria/chemicals/proteins. Keratinocytes, melanocytes, Langerhans cells, mast cells, endothelial cells, and nerve fibers cooperate in the maintenance of homeostasis.

Stress decreases proliferation of keratinocytes. Tsuchiya et al. counted the number of epidermal cells that were positive for proliferating cell nuclear antigens and found that the number was lower after immobilization (1). Intensive studies by Elias et al. on the barrier function of the cornified layer demonstrate that psychological stress decreases epidermal cell proliferation, impaires epidermal differentiation, and decreases the density and size of the corneodesmosomes (2). Altemus et al. found that psychological interview stress or sleep deprivation delays the recovery of skin barrier function (3). Melanocytes are also affected by stress. Inoue et al. measured the color of the skin and the number of dihydroxyphenylalanine-positive melanocytes and found that immobilization augmented pigmentation induced by ultraviolet light (4).

In 2008 examination of human skin biopsies by Kleyn et al. clearly demonstrated the decrease in the number of Langerhans cells after the acute interview stress (5), suggesting that stress suppresses skin immune function. Effects of stress on contact hypersensitivity reaction were reported by Mettrop and Visser as early as 1971 (6). Since then, the effects of stress on the immune function of skin have been studied mostly using allergic contact hypersensitivity. In some reports, reactions were suppressed by stress but stress augmented these reactions in other reports. The controversy was explained, at least partially, by Dahbhar et al (7) and Flint et al (8). The timing, intensity, and duration of stress influence the effects of stress. The recent report by Klein et al.(5) may offer one explanation.

Hair loss might be one of the symptoms of stress reaction. Studies by Paus et al. suggested a possible relationship between stress and hair loss, based on finding an increased number of hair follicles containing apoptotic cells following the application of sonic stress (9). Substance P (10), NGF (11), neurokinin 1 (12) and mast cells (13) were reported to be mediators of the interaction. Down-regulation of lipogenesis by stress was also reported. The incorporation of 14C-acetate to sebaceous glands was decreased by about 50% after immobilization (14).

Mechanisms of the effects of stress on skin functions

Nerve system, endocrine system, and immune system function in coordination to maintain homeostasis in the body. Physiological and psychological inputs are processed in the brain together with information from memory. In order to cope with these problems the brain directs each organ to initiate defensive actions, i.e. stress reaction.

The hypothalamus-pituitary-adrenal gland axis (HPA axis) is the main pathway of stress reaction. CRH secreted from the hypothalamus stimulates the secretion of ACTH from the pituitary gland. ACTH induces the secretion of glucocorticoids from the adrenal cortex. This chain reaction provides negative feed back for the suppression of CRH secretion by glucocorticoids. Those hormones are detected in the skin (15) and considered triggers of the effects of stress on the skin. Impairment of the barrier function by stress is prevented by the cortisol antagonist RU418 (2). The involvement of ACTH in the augmentation of UV-induced pigmentation has been suggested (4).

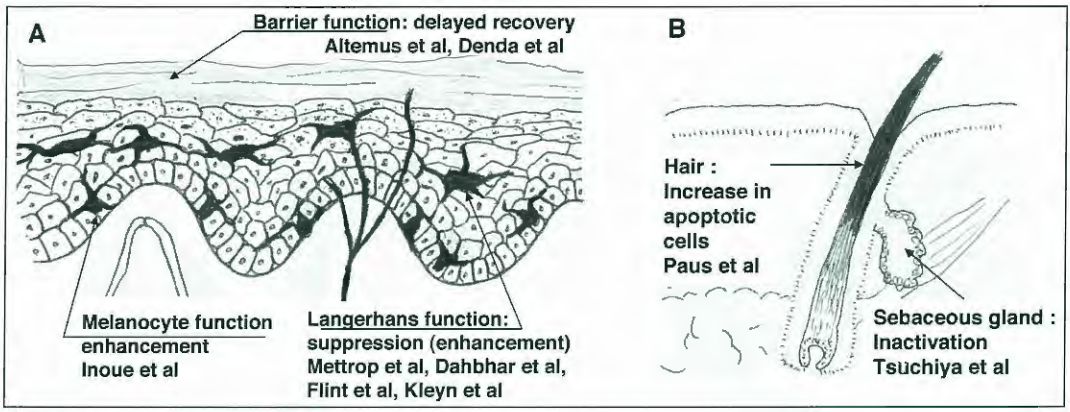

Fig. 1 Effects of stress on various skin functions.

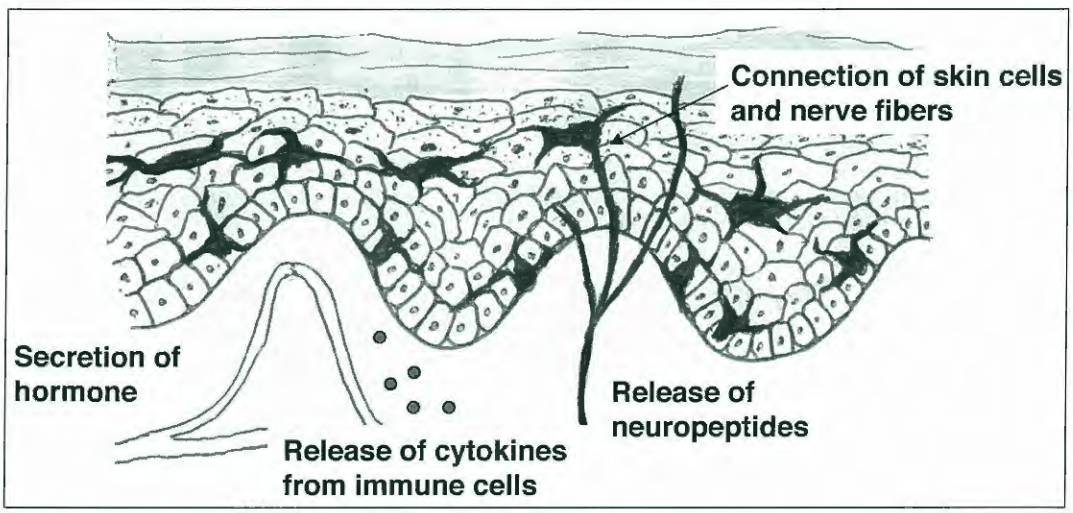

Fig. 2 Mediators of the effects of stress on the skin.

Involvement of nerve-related factors has also been suggested. The skin is a heavily innervated organ. It was previously thought that nerve fibers end at the bottom of the epidermis following observation of silver staining of the skin sections, but the advances in the methods and equipment used for immunohistochemistry and electron microscopy have demonstrated that free nerve endings enter the epidermis and even extend into the cornified layer. Several neuropeptides are released from nerve fibers in the skin, such as CGRP and substance P. We demonstrated an intimate association between epidermal Langerhans cells and nerve fibers (Fig.3A, 16). The antigen-presenting function of Langerhans cells was suppressed by CGRP (Fig.3B, 16).

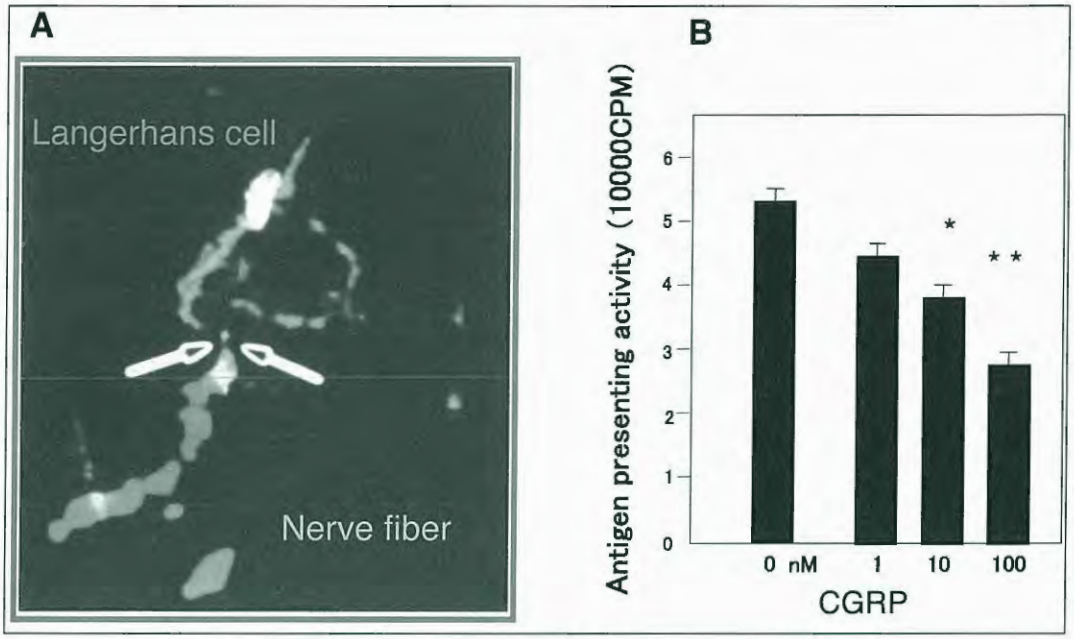

Fig. 3 A, Intimate association of nerve fibers with a Langerhans cells. B, Suppression of Langerhans cell function by the neuropeptide, CGRP. (Hosoi et al Nature 1993).

The most peripheral organ is connected with central nerve system. Kleyn et al. took biopsies before and after acute social stress and demonstrated that Langerhans cells were decreased and that the CGRP level was increased after stress (5). Catecholamines have been reported to regulate Langerhans cell function (17). Another neuropeptide, substance P, is increased in response to immobilization stress (18). Paus et al. hypothesized that substance P is a regulator of stress reactions affecting the hair cycle (13). In a previous report we summarized various effects of neuropeptides on the skin (19).

The nervous system, endocrine system, and cutaneous immune system cooperate to maintain homeostasis of the skin. Nerve-related factors regulate skin immune cells (20), and immune factors stimulate the extension of nerve fibers (21). Hormones are well known to regulate immune functions. Those 3 systems interact with each other and regulate the skin functions. Based on these data, a group of Harvard scientists called this system the "NICE network" or neuro-immuno-cutaneous endocrine network (Fig. 4; 22, 23).

Regulation of the skin condition by cosmetics via the NICE system

The data presented above suggest that a systemic approach could be as effective as topical treatment. Some basic studies support the idea of the use of odorants for the protection of the skin from the stress.

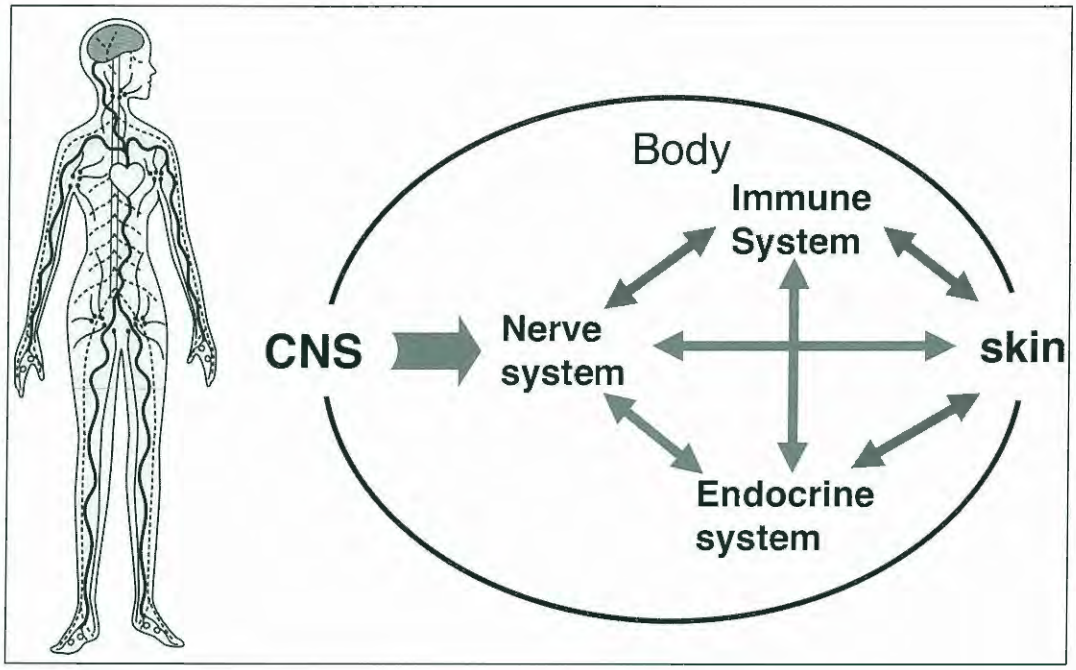

Fig. 4 NICE network.

The allergic contact hypersensitivity reaction suppressed by stress was prevented by inhalation of certain types of odorants (24). Interestingly, other types of odorants upregulate the reaction (25). The barrier function of the skin was also influenced by odorant inhalation (26). These findings support the utilization of odorants for skin care.

First, the effect of mental stress on skin was examined. When volunteers inhaled the odorant (dimethoxymethylbenzen) chosen for suppression of the induction of serum cortisol (Fig.5), impairment of barrier recovery was blocked (Fig 6). In this experiment, the stress reaction was induced by color word Stroop test.

Utilizing the odorant and other ingredients, we developed a system of cosmetics. The effects of daily use of skin care products were examined. Subjects were asked to use their own cosmetics or the newly developed skincare system on their face for one month.

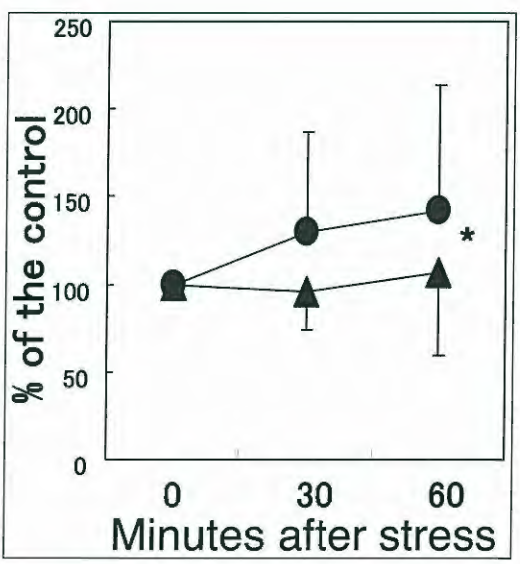

Fig. 5 Suppression of stress-induced serum cortisol. Twenty volunteers underwent the color word Stroop test with (5) or without(l) odorant inhalation.

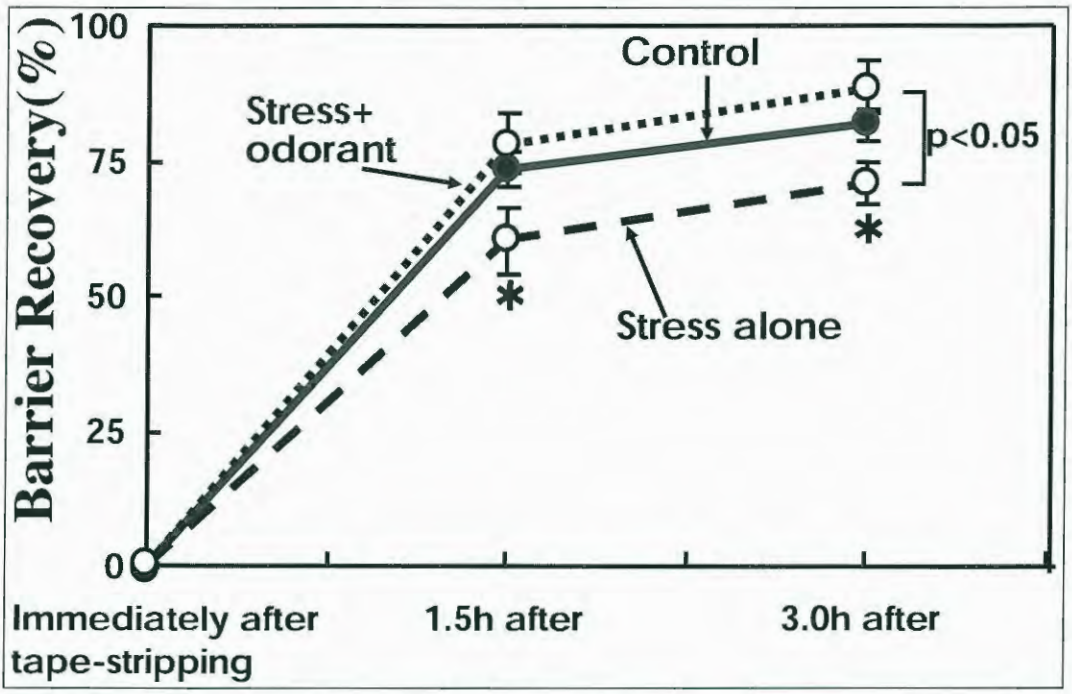

Fig. 6 *Stress-induced impairment of the barrier function was blocked by inhalation of odorant. Transepidermal water loss was measured before and after tape stripping and the recovery rate was calculated. n=20.*

They were also told not to use skin care cream on their arms. The moisture content of skin was measured by conductance and was increased in the subjects who used the novel skin care system, but decreased in the group using conventional cosmetics (Fig 7). From the data obtained after repeated use of the novel cosmetics (Fig. 8), the usefulness of applying the NICE approach to cosmetics was confirmed.

We then extended further the idea of applying NICE approach. The human body has a potent ability to manage stress reactions. Our next aim was to facilitate the self-defense system, rather than simply block mal-function. Dehydroepiandrosterone (DHEA) is secreted from adrenergic tissue. DHEA is known to decrease as people age. Various functions of DHEA have been reviewed by Oberbeck et al. (27), including regulation of metabolism, cardiovascular function, and immune function. DHEA was increased after acute and chronic stress and is supposed to contribute to the protection of the body (28). Following is the summary of the work taking advantage of the protective effect of DHEA (29).

We developed a fragrance and examined its effect by measurement of contingent negative variation (CNV) on electroencephalogram. CNV is considered an index of psychological tension. The fragrance, containing mimosa, rose, and violet leaf, appeared to be beneficial (Fig. 9).

Then, the usefulness of the fragrance in cosmetic products for daily use was examined with 90 young women. Changes in DHEA levels in saliva were measured by radioimmunoassay and the values are shown in Fig 10.

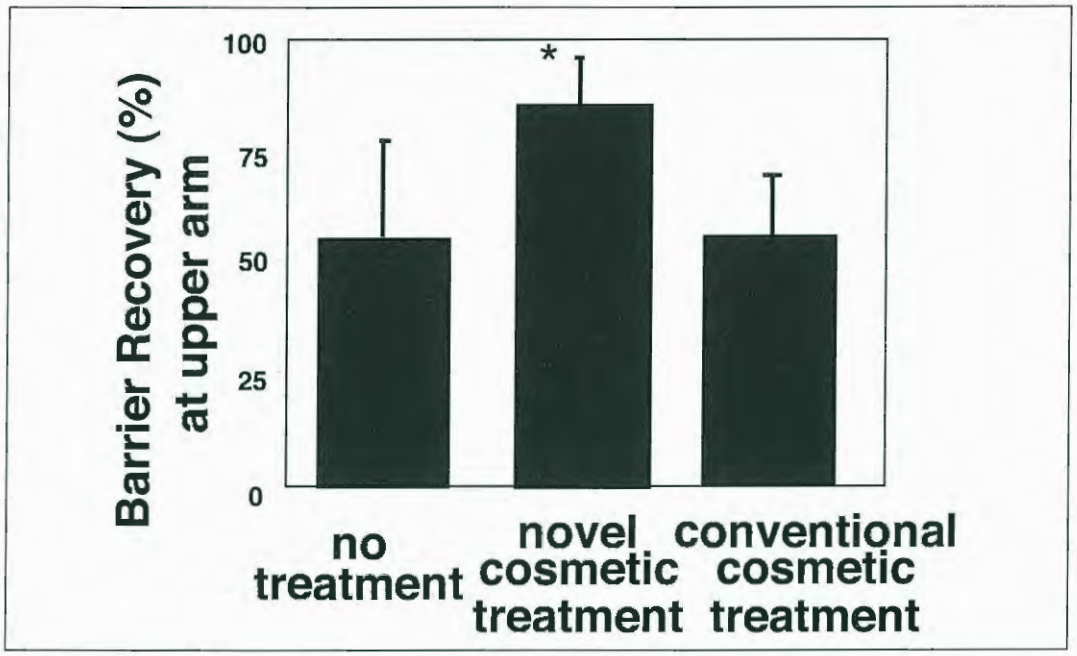

*Fig. 7 Improvement of the barrier function at upper arm by a novel cosmetic treatment. n=20, *p<0.05.*

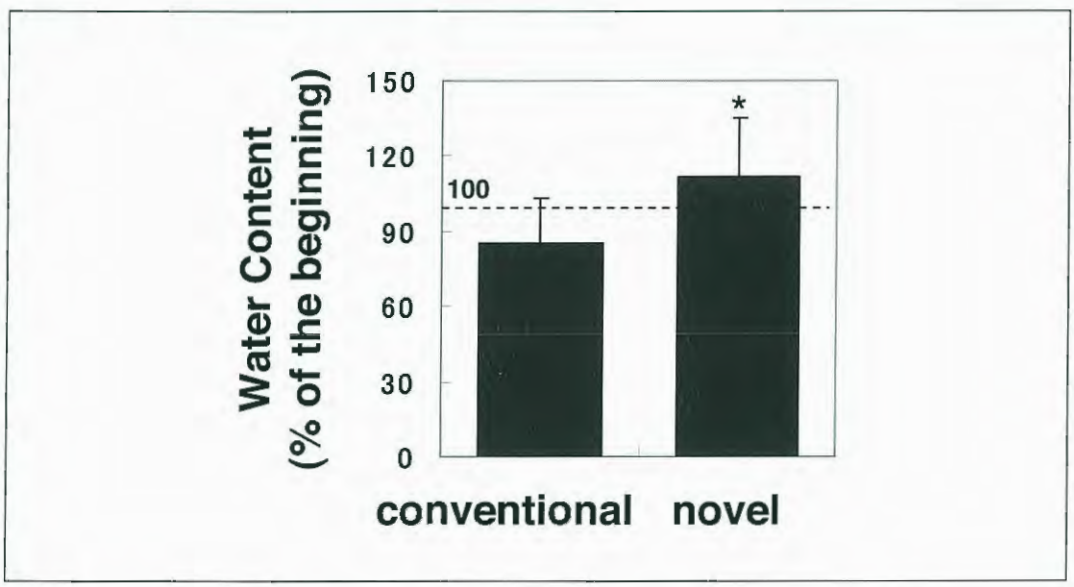

*Fig. 8 Changes in the moisture content of the skin measured by skin conductance on the arm n=20, *p<0.05.*

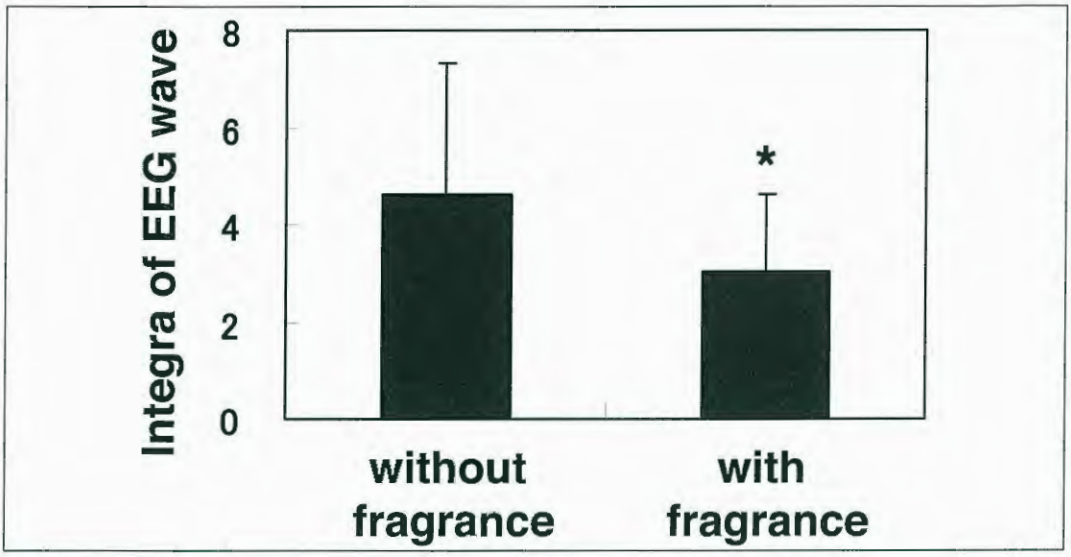

*Fig. 9 Suppression of Contingent negative variation measured on an electroencephalogram. n=8, *p<0.05.*

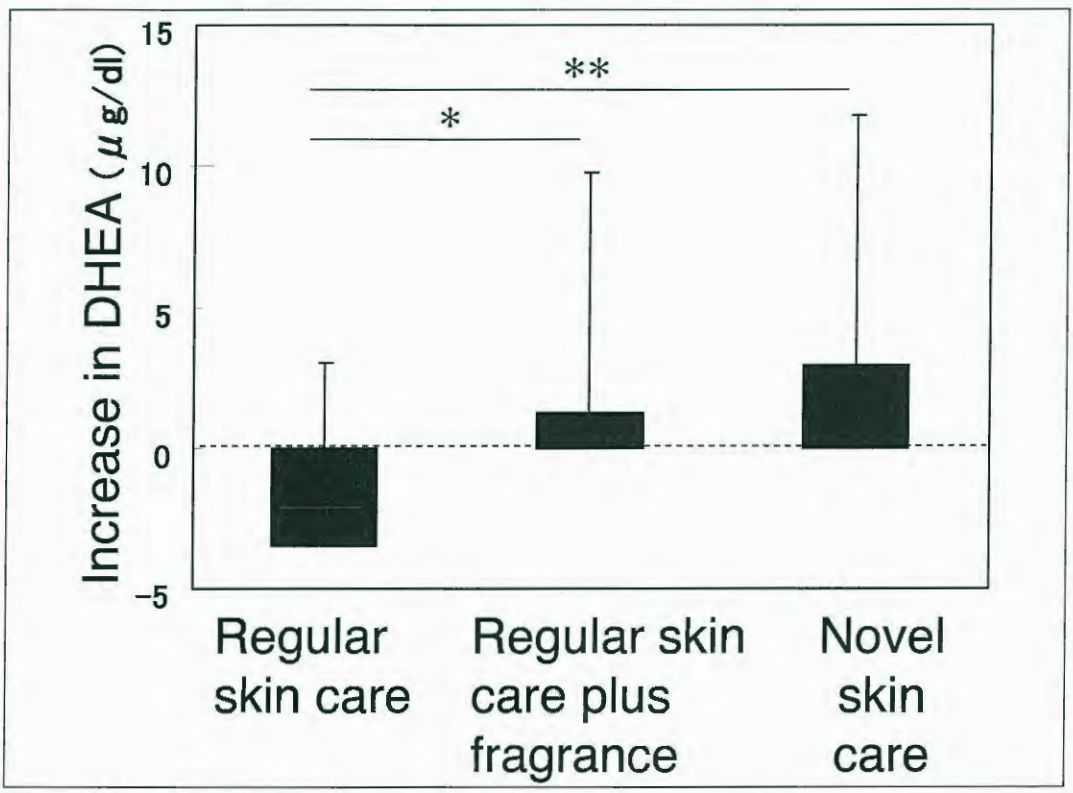

*Fig. 10 Increase in salivary DHEA by specially designed fragrance or skin care. n=30, *p<0.05, **p<0.01.*

Compared to the decrease in Group 1 who continued to apply their own skin care products on their face, a slight but significant increase was detected in Group 2 who used the fragrance we developed as an environmental scent and in Group 3 who used the specially designed skin care system. These findings demonstrate that daily use of certain cosmetics or exposure to an ambient fragrance effectively enhances the circulation of DHEA.

Under these conditions moisture content of the skin (skin conductance) was measured on the forearm where no cosmetic treatment was applied during the experiment. Fig. 11 shows the changes in moisture content from the beginning of the experiment to the end. In Group 1, the moisturer content was decreased after one month, suggesting that the skin became dried without treatment. However, there was no such decrease in Group 2 and Group 3. These findings suggest that certain fragrances or skin care products potently protect the skin by enhancing the self-protective mechanisms.

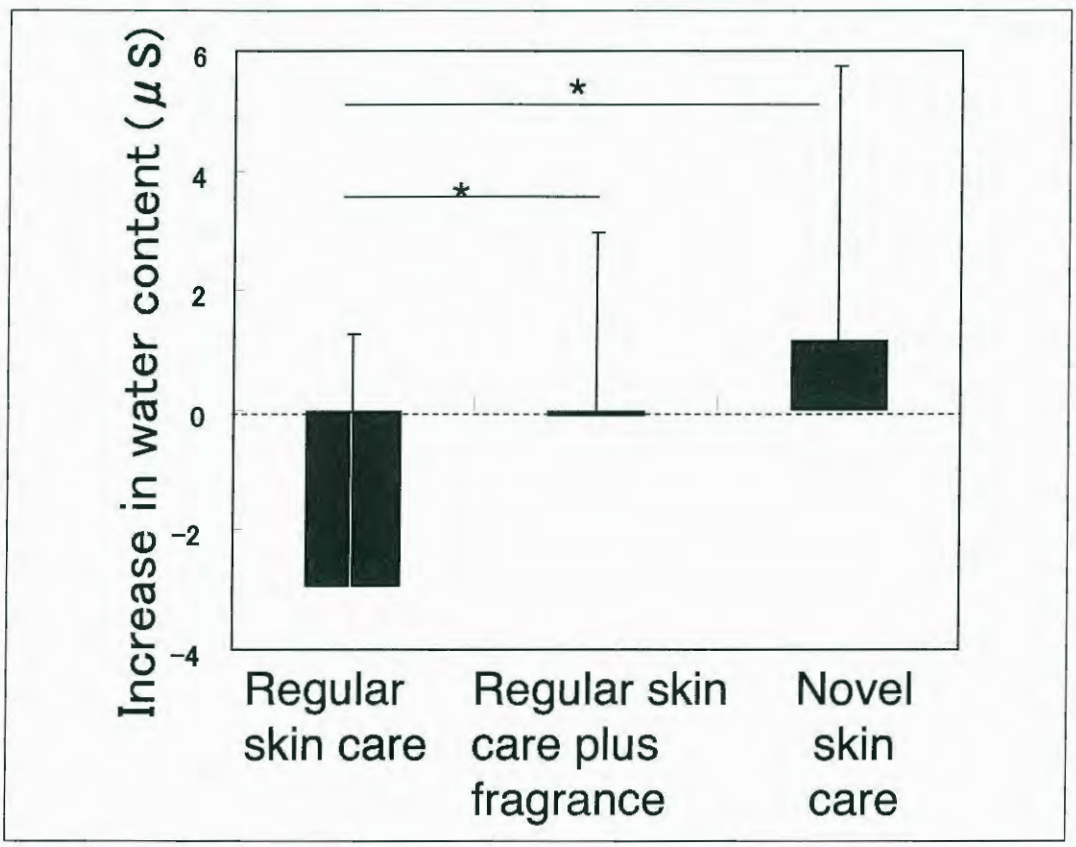

Fig. 11 *Increase in moisture content of the arm in response to specially designed fragrance or skin care products. n=30, *p<0.05, **p<0.01.*

CONCLUSION

Intensive studies have provided evidence of the relation of the skin to the body and mind, indicating the effects of the NICE network. Skin care designed to activate the NICE network is a novel approach. Not only the topical application of cream or lotion but also the approaches to activating the internal system seem to be effective. Dermatologists in Yokohama City University demonstrated that the use of a fragrance improved the severity score of itchy atopic dermatitis (30). I hope that further information in this field is accumulated by nice network of scientists from east and west, resulting in the evolution of superior cosmetics.

ACKNOWLEDGEMENTS

The establishment of the NICE system and its application to cosmetics are described above. These works were done by a great many people and I would like to thank all of them, especially Dr. Granstein, Dr. Asahina, Dr. Tsuchiya and Dr. Haze.

References

1) **Tsuchiya T, Horii I. (1996)** Epidermal cell proliferative activity assessed by proliferating cell nuclear antigen decreases following immobilization-induced stress. *Psychoneuroendocrinol.* **21:** 111-117.
2) **Choi EH, Brown BE, CrumrineD, Chang S, Man MQ, Elias PM, Feingold KR. (2005)** Mechanisms by which psychologic stress alter s cutaneous permeability barrier homeostasis and stratum corneum integrity. *J. Invest. Dermatol.* **124:** 587-595.
3) **Altemus M, Rao B, Dahbhar FS, Ding W, Granstein RD (2001)** Stress-induced changes in skin barrier function in healthy women. *J. Invest. Dermatol.* **117:** 309-317.
4) **Inoue K, Hosoi J, Ideta R, Ohta N, Ifuku O, Tsuchiya T. (2003)** Stress augmented ultraviolet-irradiation-induced pigmentation. *J. Invest. Dermatol.* **121:** 165-171.
5) **Kleyn CE, Schneider L, Saraceno R, Mantovani C, Richards HL, Fortune DG, Cumberbatch M, Dearman RJ, Terenghi G, Kimber I, Griffiths CE (2008)** The effects of acute social stress on epidermal Langerhans' cell frequency and expression of cutaneous neuropeptides. *J. Invest. Dermatol.* **128:** 1273-1279.
6) Mettrop PJ, Visser P. (1971) Influence on the induction and elicitation of contact dermatitis. *Psychophysiology* **8:** 45-53.
7) **Dbabhara FS, MacEwen BS. (1997)** Enhancing and suppressive effects of stress hormones on skin immune function. *Brain Behav Immunol.* **11:** 286-306.
8) **Flint MS, Valosen JM, Johnson EA, Miller DB, Tinkle SS. (2001)** Restraint stress applied prior to chemical sensitization modulates the development of allergic contact dermatitis differently than restraint prior to challenge. *J. Neuroimmunol.* **113:** 72-80.
9) **Arck PC, Handjiski B, Hagen E, Joachim R, Klapp BF, Paus R. (2001)** Indications for a "brain-hair follicle axis (BHA)": inhibition of keratinocyte proliferation and up-regulation of keratinocyte apoptosis in telogen hair follicles by stress and substance *P. FASEB J.* **15:** 2536-2538.
10) **Arck PC, Handjiski B, Peters EM, Peter AS, HagenE, Fischer A, Klapp BFl, Paus R (2003)** Stress inhibits hair growth in mice by induction of premature catagen development and deleterious perifollicular inflammation events via neuropeptide substance P-dependent pathways. *Am. J. Pathol.* **62:** 803-814.
11) **Peters EM, Handjiski B, Kuhlmei A, Hagen E, Biclas H, Braun A, Klapp BF, Paus R, Arck PC. (2004)** Neurogenic inflammation in stress-induced termination of murine hair growth is promoted by nerve growth factor. *Am. J. Pathol.* **165:** 259-271.
12) **Arck PC, Handjiski B, Kuhlmei A, Peters EM, Knackstedt M, Peter A, Hunt SP, Klapp BF, Paus R. (2005)** Mast cell deficient and neurokinin-1 receptor knockout mice are protected from stress-induced hair growth inhibition. *J. Mol. Med.* **83:** 386-396.
13) **Peters EM, Arck PC, Paus R. (2006)** Hair growth inhibition by psychoemotional stress: a mouse model for neural mechanisms in hair growth control. *Exp. Dermatol.* **15:** 1-13.
14) **Tsuchiya T, Horii I. (1994)** Different effects of acute and chronic immobilization stress on plasma testosterone levels. *Psychoneuroendocrinol.* **20:** 95-102.
15) **Slominski AT, Botchkarev V, Choudhry M, Fazzl N, Fechner K, Furkert J, Krause E, Roloff B, Sayeed M, wei E, Zbytek B, Zipper J, Wortsman J, Paus R. (1999)** Cutaneous expression of CRH and CRH-R. Is there a "Skin stressresponse system?" *Ann. NY Acad .Sci.* **885:** 287-311.
16) **Hosoi J, Murphy GF, Egan CL, Lerner EA, Grabbe S, Asahina A, Granstein RD. (1993)** Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature* **363:** 159-163.

17) **Seiffert K, Hosoi J, Torii H, Ozawa H, Ding W, Campton K, Wagner JA, Granstein RD. (2002)** Catecholamines inhibit the antigen-presenting capability of epidermal Langerhans cells. *J. Immunol.* **168:** 6128-6135.

18) **Hosoi J, Tanida M, Tsuchiya T. (2003)** Regulation of plasma substance P and skin mast cell s by odorants. *J. Cutan. Med. Surg.* **7:** 287-291.

19) **Peters EM, Ericson ME, Hosoi J, Seiffert K, Hordinsky MK, Ansel JC, Paus R, Scholzen TE. (2006)** Neuropeptide control mechanisms in cutaneous biology: physiological and clinical significance. *J. Invest. Dermatol.* **126:** 1937-1947.

20) **Torii H, Tamaki K, Granstein RD. (1998)** The effects of neuropeptides/hormones on Langerhans cells. *J. Dermatol. Sci.* **20:** 21-28.

21) **Torii H, Yan Z, Hosoi J, Granstein RD. (1997)** Expression of neurotrophic factors and neuropeptide receptors by Langerhans cells and the Langerhans cell-like cell line XS52: further support for a functional relationship between Langerhans cells and epidermal nerves. *MISSING REVIEW TITLE* **109:** 586-591.

22) **O'Sullivan RL, Lipper G, Lerner EA. (1998)** the neuro-immuno-cutaneous-endocrine network : relationship of mind and skin. *Arch Dermatol* **134:** 1431-1435.

23) **Brazzini B, Ghersetich I, Hercogova J, Lotti T. (2003)** the neuro-immuno-cutaneous-endocrine network. Relationship between mind and skin. *Dermatol Ther* **16**: 123-131.

24) **Hosoi J, Tanida M, Tsuchiya T. (2001)** Mitigation of stress-induced suppression of contact hypersensitivity by odorant inhalation. *Br. J. Dermatol.* **145:** 716-719.

25) **Hosoi J, Tsuchiya T. (2000)** Regulation of cutaneous allergic reaction by odorant inhalation. *J. Invest. Dermatol.* **114:** 541-544.

26) **Denda M, Tsuchiya T, Shoji K, Tanida M. (2000)** Odorant inhalation affects skin barrier homeostasis. *Br. J. Dermatol.* **142:** 1007-1010.

27) **Oberbeck R and Kobbe P. (2010)** dehydroepiandrosterone(DHEA): a steroid with multiple effects. Is there any possible option in the treatment of critical illness? *Curr. Med. Chem.* **17:** 1039-1047.

28) **Maninger N, Capitanio JP, Mason WA, Ruys JD, Mendoza SP. (2010)** Acute and chronic stress increase DHEAS concentrations in rhesus monkeys. *Psychoneuroendocrinology* Feb 11 E-pub.

29) **Hosoi J, Haze S, Gozu Y, Sakai K, Moriyama M, Tsuchiya T. (2006)** Stimulation of DHEA secretion and regulation of skin functions by odorants or skin care. *The Autonomic Nervous System* **43:** 375-379.

30) **Hariya T, Kobayashi Y, Aihara M, Ishiwa M, Shibata M, Ichikawa H, Ikezawa Z. (2002)** Effects of sedative odorant inhalation on patients with atopic dermatitis. *Arerugi* **51:** 1113-1122.

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Sichuan pepper as a skin "spice"

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Summary

Zanthoxylum bungeanum Maxim is a perennial plant endemic to the Sichuan province of China. Traditionally, it is employed as a culinary spice for its unique tingling-inducing properties, bound to the presence of a complex mixture of alkamides, and especially hydroxy-α-sanshool. Alkamides have a multi-receptor binding profile, being capable to interact with a host of ionotropic- (calcium-, potassium- and sodium type) and metabotropic- (peripheral cannabinoid receptors, CB2) receptors, and are responsible for both the chemestetic and the analgesic properties of the plant, known as the "toothache tree" for its use in folk dentistry.

Apart from the dietary uses, the pericarps of the seeds of *Z. bungeanum* are the source of an oily extract used in dermatology and in cosmetic formulations to reduce itching and skin discomfort on sensitive and challenged skins. Preclinical and clinical studies have been carried out to elucidate the molecular mechanism of activity of this extract and validate its clinical use in the treatment of itching of various origin. Recently, the lipophilic extract of the pericarp of *Z. bungeanum* has also been reported to exert lifting activity, showing visible and transient improvement of facial wrinkles.

Riassunto

Lo *Zanthoxylum bungeanum* Maxim é una pianta perenne endemica della provincia cinese del Sichuan ed il suo frutto privato dei semi (pericarpo), è utilizzato come spezia culinaria per la sua capacità di indurre, grazie ad una complessa miscela di alchilamidi, in particolare l'idrossi-α-sanshoolo, una particolare sensazione di formicolio. Le alchilamidi sono in grado di interagire sia con recettori ionotropici (di tipo calcio-, potassio- e sodio-) che metabotropici (recettori periferici dei cannabinoidi e CB2) e sono responsabili delle proprietà chemestetiche ed analgesiche della pianta, nota anche come "albero del mal di denti" per il suo utilizzo nell'odontoiatria tradizionale. Oltre agli usi alimentari, i pericarpi di *Z. bungeanum* sono la fonte di un estratto oleoso impiegato, in dermatolo-

gia e in prodotti cosmetici, per ridurre la sensazione di prurito e di fastidio su pelli sensibili o danneggiate. Sono stati condotti studi pre-clinici e clinici per indagare il meccanismo d'azione di questi estratti e validarne l'uso clinico nel trattamento del prurito di varia origine. Recentemente è stato dimostrato che l'estratto oleoso del pericarpo di *Z. bungeanum* espleta anche un'attività di lifting immediato, promuovendo un miglioramento visibile e transitorio delle rughe del viso.

INTRODUCTION

The genus *Zanthoxylum* (Rutaceae family) encompasses at least 250 species (and as much synonyms) endemic to both the Old and the New World.

The Chinese Flora (1) alone describes 41 different species and varieties of this genus.

The botanical subtleties of the genus *Zanthoxylum* are compounded by the broad use of the term Sichuan pepper, that refers to a spice obtained from the fruit husks (pericarps) of various Asian *Zanthoxylum* species, all very similar in terms of colour, size, external surface, as well as sensory properties.

Within these species, *Z. bungeanum* Maxim is the best documented in the Traditional Chinese Medicine, and the Chinese Pharmacopeia (2) reports a specific monograph.

As a medicinal plant, it is employed to reduce the toothache, a use that has gained the plant the attribute of "toothache tree".

Z. bungeanum Maxim is popular because of the traditional culinary use of the fruit husks, valued for their aromatic odour and pungent effect, followed next by a sort of numbness on the tongue and the oral cavity, that is deftly exploited in the Chinese and Japanese cuisine to reduce the irritant properties of some foods, and especially hot pepper (3). Despite its pleasant and delicated citrus note, *Z. bungeanum* is famous mainly for these chemestetic properties, variously defined (tingling, paralytic pungency, electricity-mimic) (4). Tingling is a tone totally absent in the Western cuisine, being rather typical of the Far-East culinary tradition, and belongs to realm of chemestesis, namely the food-induced oral somatosensation non mediated by taste- or smell receptors (4).

Familiar chemestetic sensations are the ones induced by capsaicin from chilly pepper (heat) or menthol from peppermint (cold). Despite investigations spanning several decades, the molecular mechanism of tingling is still poorly known (4), and might actually be a composite sensation resulting from the stimulation of tactile sensors and the inhibition of sensory nerves function (see infra).

Tingling induced by the Sichuan pepper has been related to the presence of unsaturated alkamides, and especially to a pair of diastereomeric unsaturated amides (hydroxy α- and β-sanshoools, 1a and 1b, respectively) (4), for which a series of specific receptors have recently been identified and cloned. Thus, hydroxy α-sanshoools bind to and inhibit the two-pore potassium channels KCNK3, KCNK9 and KCNK18, a class of pH- and general anesthetics-sensitive ion channels. Hydroxysanshools, as well as other constituents from Sichuan pepper, can also interact with other chemesthetic TRP-receptors, and, therefore, it is not clear to what extent tingling from Sichuan pepper can be traced solely to the interaction of sanshools with two-pore potassium channels alone (5). Sanshools have been recently shown to interact, at least partially, with TRPA1 and TRPV1, and this implication suggests a possible hypotesis for the pungent sensation evoked by Sichuan pepper (6). On the other hand, the interaction with channels of the KCNK type might be involved in the regulation of thermal and physical stimuli in sensory terminals of cutaneous neurons (7).

The soothing sensation induced on the mucous oral membranes has provided a rationale to develop *Z. bungeanum* as a botanical ingredient to soothe sensitive skin. In this condition, the skin is over reactive to external stimuli and environmental factors like temperature variations, UV exposure, and cosmetic detergents (8), and a large share of the population (ca. 60% of women and 40% of men) believes having a sensitive skin and looks for suitable topical products to relief this condition (9).

FROM SICHUAN PEPPER TO THE COSMETIC INGREDIENT

A lipophilic extract from *Z. bungeanum* Maxim was prepared from the fruit pericarps by extraction and fractionation with CO_2 in hypercritical conditions (10). The process requires the maceration of the fruits and subsequent extraction with CO_2 in continuous recycling for two hours at a temperature of 45°C and a pressure of 250 bar. After CO_2 evaporation the oily fraction is dried under vacuum and standardized to contain 4.4–6.4% of isobutylamides, by dissolving it in about 80% oleyl alcohol. The final product*, hereafter referred to as Lipophilic CO_2 Extract, is absent of potentially harmful solvent and has been Ecocert® validated.

Translating ehnopharmacological documentation into scientific evidence

The traditional use of *Z. bungeanum* and preliminary studies demonstrating an interaction of the Lipophilic CO_2 Extract at the neuro-muscolar junction served as a basis for a series of studies aimed at defining the functional profile of the product and its use in cosmetic and dermatological products. Thus, the Lipophilic CO_2 Extract has been tested in vitro on the sciatic nerve-musculus extensor digitorium longus preparation of rat to evaluate its effects on synaptic transmission (11).

A Ringer solution with the addition of a detergent was used to improve the solubility of the Lipophilic CO_2 Extract, and a strong, transient action on neuromuscular synaptic transmission (increased frequency of miniature potentials and occurrence of spontaneous plate potentials) was demonstrated. The activation step led to a quick depletion of neurotransmitters, and to a subsequent block of synaptic transmission in a tetrodotoxin (TTX) (1μM) sensitive fashion. This suggested the involvement of voltage-dependent Na^+ channels, since TTX is a specific probe for this end-point, blocking its pore and function, and is in accordance with the effects on sodium and potassium exchanges across the membranes of nerve cells reported for plant alkamides (12). This, and the results obtained in the presence of d-tubocurarine, an antagonist of the nicotinic acetylcholine receptors, suggested a pre-synaptic activity for the Lipophilic CO_2 Extract, and the lack of any direct effect on the muscle (13). These observations are in substantial accordance with the results reported for thermal and tactile sensitivity on hydroxy-α-sanshool (14), and show that alkamides from *Z. bungeanum* are essentially "excitability sensitizers", lacking direct action on muscular activity but affecting sensory receptors as well as neural transmission activity.

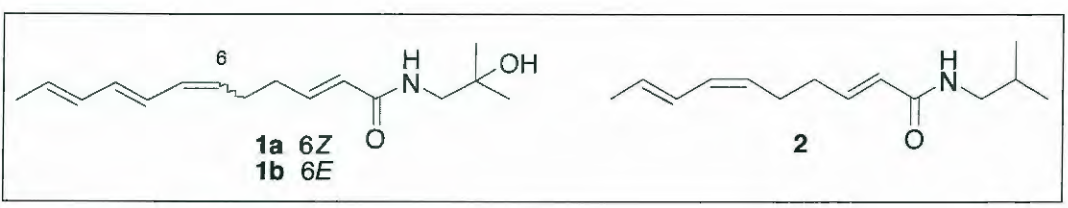

Fig. 1 Hydroxy-α-sanshool and hydroxy-β-sanshool.

* *Trade Name: Zanthalene®*

CLINICAL TRIALS

Numerous clinical trials have been carried out to demonstrate the soothing and anti-itching properties of cosmetic and topical formulations containing the Lipophilic CO_2 Extract. Even if itching might be perceived as a minor problem, it might strongly affect social life especially in cases of pathologies of the genital area or strong allergies.

Soothing effect by thermal cutaneous sensitivity[13]

A randomized, double blind study was carried on 12 volunteers, who were trained to define a heat sensation after a thermal stimulus, as "warm", "hot" and "painful" at a certain temperature. A device called Sensitherm®, with a thermal head giving regularly increasing temperature on the skin, was used throughout the experiment. An emulsion contianing 0.5% of the Lipophilic CO_2 Extract was applied to the tested area, and a blank emulsion as control. Measurements were taken before the application and 30 minutes after the application of a thermal stimulus. An increase in the sensitivity threshold was observed for 10 out of 12 volunteers. Identification of heat sensation increased by 1.2±0.4°C, warm, as "sensorial comfort", increased by 1.3±0.6°C, hot as "sensorial discomfort", increased by 1.0±0.4°C, whereas the threshold for the definition of the painful sensation did not vary. The observation that the Lipophilic CO_2 Extract affects thermal sensitivity *in vivo* is in apparent contrast with the *in vitro* observation that hydroxy-α-sanshooll increases thermal and tactile sensitivity (14), and might be due either to a desensitizing effect (after stimulation the receptor becomes incapable of functioning) or to a prevailing anesthetic effect mediated by the effect of alkamides on sensory nerve function, and, in particular, sodium and potassium exchanges across the membranes of nerve cells (12).
Remarkably, the pain perception threshold was not affected. Pain receptors or nociceptors are activated by potentially damaging stimuli, and are a heterogenous group of sensors, whose best characterized members are the thermo-TRP receptors (7). Hydroxy-α-sanshool has been reported to increase thermal sensitivity in cultured skin cells (6-8), and the paradoxical results observed on thermal sensitivity in volunteers are presumably the result of a combination of direct sensory stimulation and decreased transmission of neural information.

Soothing effect prior to the application of hair dyes[15-16]

A clinical trial was performed on twenty female volunteers with a specific sensitivity to hair dyes. Their scalp was evaluated by a professional hairdresser prior to the application of the soothing lotion (half of the scalp with a the Lipophilic CO_2 Extract containing lotion at 0.5% and half of the scalp with the blank lotion). Twenty to thirty minutes after the application of the lotion, the hair dye was applied. After dye removal, the hairdresser evaluated the scalp conditions on parameters such as dandruff, irritation, redness, etc., and the volunteers filled in a form describing their scalp discomfort during the hair. Remarkably, 85% of the volunteers chose the the Lipophilic CO_2 Extract - containing lotion when asked to assign a product preference ($p<0.0001$).
After hair dyeing, all volunteers felt discomfort in the blank treated part, whereas 70% felt no discomfort in the Lipophilic CO_2 Extract treated part and 30% felt "less discomfort" in the Lipophilic CO_2 Extract treated part ($p=0.031$). The soothing effect appeared earlier with the Lipophilic CO_2 Extract in 80% of the volunteers, with the placebo lotion in 5% of them, while in 15% of the cases no difference was perceived

(p=0.006). No variation in the scalp or hair conditions was noticed by the hairdresser. Taken together, these observations show that the Lipophilic CO_2 Extract could significantly reduce scalp discomfort in the majority of volunteers, qualifying it as a soothing active ingredient for hair care cosmetic products, like dyes, lotious, or anti-dandruff shampoos.

Anti-itching effect after mosquito bites[17]

A spray solution containing the Lipophilic CO_2 Extract at 0.5% was clinically tested to evaluate its tolerability and its soothing and anti-itching efficacy after mosquito bites.

The study involved 40 volunteers who got at least three mosquito (Aedes aegypti) bites on each arm. Immediately after the bites, half of the patients treated the bites on their right arm, the other half treated their left arm, with the second arm functioning as control.

The volunteers were required to record the intensity of itching from 1 (very strong itching) to 5 (no itching) and/or pain at 15, 30, 60 minutes and 2, 4 and 24 hours after biting.

Twenty seven out of 40 volunteers (67.5%) reported a positive effect compared to the non treated control arm, whereas no difference was detected by 13 volunteers, showing that the Lipophilic CO_2 Extract could improve the "no itching" and the "weak itching" scores, in particular at short and medium term.

Anti-itching effect on atrophic vaginitis[18]

Infections of the feminine genital apparatus like vaginitis and vaginosis are some of the most common gynaecological problems, and, in the majority of cases, are due to bacterial or fungal infections.

The main symptoms are burning, itching and mucosae inflammation. At least for *Candida* infections, these socially invalidating and discomforting symptoms might be related to the fungal metabolization of the endogenous cannabinoid anandamide, with an overall shift from the "soothing" activation of cannabinoid receptor to "burning" activation of TRPV1, the capsaicin receptor (19). In these conditions, the control of the bacterial infection and inflammation should be associated to a reduction of itching, so as to improve the compliance of the anti-infective therapy. The soothing effect of the Lipophilic CO_2 Extract in atrophic vaginitis was investigated in a clinical study on 15 female patients diagnosed with atrophic vaginitis.

Both objective and subjective symptoms were used as the end-point, and the severity of observed and reported symptoms (itching, burning and dryness in the atrophic vaginitis) was assessed according to the Scott-Huskisson rating scale.

Two multicomponent topical products (a vaginal cream which was associated to an intimate cleanser) containing the Lipophilic CO_2 Extract along with other active (anti-inflammatory and/or antibacterial) ingredients were used. Apart from the Lipophilic CO_2 Extract, the products tested contained standardized extracts from *Glycyrrhiza glabra, Matricaria chamomilla, Curcuma longa,* and *Malaleuca alternifolia* (Zantogin® Cream and the Zantogin® Detergente from Humana Pharma, Milan, Italy), and were applied twice daily (cream, morning and evening) and used twice daily (detergent) for 10 days.

Controls were taken before the beginning of the treatment, at the end of the ten days' treatment as well as 7 days from the termination of the treatment.

At the end of the treatment, symptoms as itching and burning disappeared in 100% ($p<0.01$) of the patients, whereas the "dryness" parameter decreased by 85% ($p<0.05$) as reported in Table I.

These subjective reports were confirmed by the investigators' objective observation, who found a general improvement in the mucosae conditions compared to the hyperhaemic and hypertrophic state at the beginning of the trial.
Clearly, the multicomponent topical products had effectively counteracted the irritant symptoms typical of the disease, exerting an important anti-itching activity that can be reasonably ascribed to the Lipophilic CO_2 Extract, since none of the other ingredients have been reported to direct affect this end-point. Although the number of patients was small, and the study was limited to a short period of time (10 days), its results were highly stastistically significant.

Anti-itching and soothing effect in the treatment of psoriasis [20]

Psoriasis is a form of relapsing, non-infective, chronic, autoimmune dermatitis widespread in all human races but more common in Caucasians and most frequent in puberty and menopause.
The main dermatological symptoms are papules and erythematous plaques covered with scales. Although itching is not always present, in some cases it is so evident that it may evolve to more severe forms of dermatological disorders due to the patient's attempt to relieve it.
From a histological standpoint, psoriasis lesions are hyper-proliferative areas with an epidermis turnover that might be 5 to 10 times faster than normal skin.
In consideration of the significant pro-inflammatory and pro-immune role played by some mediators in the pathogenesis of psoriasis, a multicomponent topical preparation has been developed and clinically tested. The active ingredients of this preparation (Psoribiox® (Montefarmaco OTC, Bollate, Italy) were 18-β-glycyhrretinic acid phytosome®, boswellic acids, the Lipophilic CO_2 Extract, a peptidic-proteic zebrafish egg derivative, and 7-dehydrocholesterol (pro-Vitamin D).
The study was a three-arm study, with twenty patients in each study arm. The patients were treated, respectively with: a) a formulation endowed with antiproliferative activity (calcipotriol), b) a combination of this formulation and a topical product containing a zebrafish eggs extract and 7-dehydrocholesterol, and c) a combination of the calcipotriol formulation and containing 18-β-glycyrrhetic acid Phytosome® (1.0%), boswellic acids (1.5%) and the Lipophilic CO_2 Extract (1%), as well as the peptidicproteic derivative of zebrafish eggs (0.2%) and 7-dehydrocholesterol (0.005%) also used in Group B.
The study, an open single blind trial, involved a protocol with the applications of the preparations twice daily (morning and evening) for one month.

TABLE I

SYMPTOM	T=0	T=10	T=17
Itching	6.2±2.2	0*	0*
Burning	7.1±2.1	0*	0*
Dryness	8.8±1.9	1.6±0.2**	1.4±0.2**

*Symptoms performance (visual analogue scale according to Scott and Huskisson (score 0-10)) on subjects (n = 15) with a diagnosis of atrophic vaginitis before starting the treatment (T=0), after 10 days of applications (T=10) and at day 17th (T=17), 7 days after the last application of the formulations * $p < 0.01$. ** $p < 0.05$.*

The response to the different topical treatments was assessed using the visual analogue scale according to Scott&Huskisson's model (clinical scores from 0 to 10 depending on the intensity). This scale evaluates itching, erythema and desquamation.

The parameter of patch extension was objectively assessed through the instrumental measurement of the average of the two (longer and shorter) diameters of a sample consisting of at least 5 patches selected at t=0. At the end of the treatment, the multicomponent preparation containing the Lipophilic CO_2 Extract (group C) had reduced itching by 74%, erythema by 73.3%, desquamation by 64.7% and patch extension by 30% (Table II).

The three treatments reduced symptoms and improved each of the considered parameters. While calcipotriol played a major role in the observed improvement, the adjuvant therapy of group C further improved the clinical outcome, reducing itching, erythema and desquamation and slowing down the patch progress. The additional treatment also played a critical role in improving, in group C, the overall tolerability of the calcipotriol treatment.

TABLE II

PARAMETER	GROUP		
	A	B	C
Itching	2.7±0.4*	2.4±0.4*	2.0±0.2**
Erythema	3.0±0.5*	3.0±0.6*	2.0±0.2**
Desquamation	3.3±0.6*	3.2±0.5*	2.3±0.4*
Patch Extension	3.7±1.3	3.5±1.0	3.5±1.2
Efficacy (number of patients)	Excellent (6)	Excellent (6)	Excellent (14)
	Good (10)	Good (10)	Good (6)
	Poor (3)	Poor (4)	Poor (0)
Tolerability (number of patients)	Excellent (0)	Excellent (0)	Excellent (14)
	Good (19)	Good (20)	Good (6)
	Poor (0)	Poor (0)	Poor (0)

Effects of 15-day treatment (t=15) on symptoms according to Scott & Huskisson, patch extension (cm ± SD), assessment of treatment efficacy, tolerability and side effects in patients affected by slight/moderate psoriasis.

**p<0.05 vs t=0; **p<0.01 vs t=0*

Treatment: A: calcipotriol; B: calcipotriol and formulation with zebrafish and 7-dehydrocholesterol; C: calcipotriol and multicomponent formulation containing the Lipophilic CO_2 Extract

Lifting effect[21]

the Lipophilic CO_2 Extract has been extensively investigated as a skin-soothing and anti-itching agent (13, 16-18, 20), but due to its activity on voltage dependent Na^+ channels, it could, in principle, also exert lifting activity on the periocular area. A specific study evaluated the short and long term anti-wrinkle efficacy and the ability of improving skin elasticity of two different creams containing the Lipophilic CO_2 Extract creams (1% and 2%) in comparison to the same emulsion containing a reference product (spilanthol, 2) having similar sensory properties.The investigated formulations had the following composition: Cream A: 2% reference product; Cream B: 2% of the Lipophilic CO_2 Extract; Cream C: 1% of the Lipophilic CO_2 Extract.

Each cream was tested on 14 female volunteers who applied two of the three products (one on each half face) for 4 weeks. Instrumental evaluations of skin roughness by objective measurement of Ra (mean roughness) and Rz (maximum roughness value), were performed at the beginning of the test, after 2 weeks of use and at the end of the test. In order to check the short term efficacy (lifting effect), a double check at 30 minutes after single application both at the basal time and at the final control has been performed. The volunteers were also required to report their evaluation of the products by filling a detailed questionnaire on the efficacy and tolerability of the formulations. The Ra values (medium roughness parameter) of subjects applying the 1% Lipophilic CO_2 Extract cream decreased 30 minutes after the first application from 16.07 (± 4.24) to 13.45 (± 3.09), and a similar decrease (from 16.47 (± 4.34) to 15.26 (± 4.7)) was measured also between the last application and the last measurement.

Formulation A and Formulation B did not have any significant effect on the measured parameters.

The Rz parameter showed a decreasing trend from 74.74 to 68.33 (± *14.81*), although the result was not statistically significant, in the group applying the 1% Lipophilic CO_2 Extract, whilst Formulation A and B did not influence the measured parameter. With products having marked sensory properties, subjective self-evaluations can be biased by emotional feeling, but it is interesting to remark that, while Formulation A was rated as very-to fairly active for an immediate lifting effect by very-to-fairly 85.7% of the volunteers, and Formulation B by 78.6% of them, 100% of the volunteers rated in this way the 1% CO_2 *Zanthoxylum bungeanum* extract. These data are the first demonstration of a lifting activity for hydroxylated alkamides. Although not quite as potent or long-acting as Botulinum toxin, CO_2 *Zanthoxylum bungeanum* extract fully qualifies as a functional cosmetic ingredient for the temporary improvement of skin wrinkles (23).

CONCLUSIONS

The fruits husks of *Z. bungeanum* (Sichuan pepper) are an important ingredient of the Eastern cuisine. Their unique sensory properties and their use as an anti-itching and lenitive ingredient have stimulated the development of an alkamide-rich lipophilic extract from this spice CO_2 *Zanthoxylum bungeanum* extract as a skin-soothing agent (13, 16-18, 20).

The soothing and anti-itching activity of the Lipophilic CO_2 Extract has been demonstrated in controlled trials, that showed its efficacy as a natural active ingredient for the management of skin discomfort of various origins (itching from mosquito bites, itching due to inflammation or psoriasis, temperature induced skin discomfort, etc.). The thermal sensation (warming, cooling or a combination of both) observed with the Lipophilic CO_2 Extract could be due to activation of specific thermoTRPs (TRPV1 and

TRPA1, respectively), and although Sanshools show only marginal vaniloid activity (6), other constituents of the extract might interact more potently with these end-point or with other thermo-TRPs.

The rationale for investigating the relaxant activity of a shanshool-rich lipophilic extract CO_2 *Zanthoxylum bungeanum* extract on skin superficial muscles was its inhibitory activity against synaptic transmission (11), a type of activity typical of the anti-wrinkle agent Botulinum toxin (Botox®).

Regarding the possible molecular bases for the lifting activity, sanshool could relax subcutaneous muscles with an essentially ionotropic mechanism, related to interaction with sodium channels (tetrodotoxin-like activity), GABA-A receptors (benzodiazepines-like activity), or two-pore potassium channels (general anestetics-like activity) (6), and studies are underway to elucidate the relative contribution, if any, of these mechanisms to the observed activity.

Even though the molecular mechanism(s) of the culinary and medicinal uses of Sichuan pepper and of the clinical activities of its lipophilic extracts are unclear or even paradoxical in the light of the action on sensory receptors of hydroxy-α-sanshool, the Lipophilic CO_2 Extract represents, nevertheless, a clinically-validated ingredient for the management of skin itching and for the temporary improvement of skin wrinkles.

Improvements in these two conditions can easily be assessed by users, and this has undoubtedly contributed to the success of the Lipophilic CO_2 Extract as a "spice" for the skin.

References

1) **Wu ZY, Raven PJ. & Hong DY. (2008)** Flora of China. Vol. 11 (Oxalidaceae through Aceraceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
2) **Pharmacopoeia of the PR of China vol 1, 177 (2005).**
3) **McGee H. (2004)** On Food and Cooking: The Science and Lore of the Kitchen, Scribner, New York.
4) **Xiong Xiong D, Shi D, Yamamoto H, Mizuno M. (1997)** Alkamides from pericarp of *Zanthoxylum* bungeanum. *Phytochemistry,* **46:** 1123-1126.
5) **Bautista DM. et al. (2008)** Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. *Nat. Neurosci.,* **11(7):** 772-9.
6) **Riera CE. et al. (2009)** Compounds from Sichuan and Melegueta peppers activate, covalently and non-covalently, TRPA1 and TRPV1 channels. *Br. J. Pharmacol.,* **157(8):** 1398-409.
7) **Lennertz RC. et al. (2010)** Physiological basis of tingling paresthesia evoked by hydroxy-alpha-sanshool. *J. Neurosci.,* **30(12):** 4353-61.
8) **Primavera G. and Berardesca E. (2005)** Sensitive skin: mechanisms and diagnosos, *Int. J. Cosm. Sci.,* **27:** 1-10.
9) **Saint Martory C, Roguedas Contios AM, Sibaud D, Degouy A, Schmitt AM, Misery L. (2008)** Sensitive skin is not limited to the face, *Br. J. Dermatol.,* **158:** 130-133.
10) **Bombardelli E, Gabetta B. (2000)** WO00/02570, "Extracts of *Zanthoxylum* bungeanum, pharmaceutial and cosmetic formulations containing them", 20 Jan 2000.
11) **Università di Urhino, Italy, Data on file (1995).**
12) **Rotherham LW. and Semple JE. (1998)** A practical and efficient synthetic route to dihydropipercide and pipercide, *J. Org. Chem.,* **63:** 6667-6672.
13) **Cristoni A. et al. (1999)** Cosmetic application of hypercritical CO_2 plant extracts, In Conference proceedings of In-Cosmetics 1999, Paris La Defense – Verlag für Chemische Industrie, H Ziolkowsky GmbH ed, pp. 342-353.
14) **Bryant BO, Mezine I. (1999)** Alkylamides that produce tingling paresthesia activate tactle and thermal trigeminal neurons, *Brain Res.,* **25;842(2):** 452-60.
15) **Cristoni A, Artaria C. (2007)** *Zanthoxylum* alatum purified extracts reduces skin irritation, *Cosmetics,* **3:** 38-41.
16) **Data on file, Dermscan report 99827, March 2000.**
17) **Rudin W. (2002)** Evaluation of the soothing and anti-itching efficacy of Zanthalene after mosquioto bites, **Data on file.**
18) **Di Pierro F, Candidi C, Alfonso R. (2009)** Efficacia clinica di terapie combinate multicomponente a base di Krameria o *Zanthoxylum* nelle vulvovaginiti infettive, atrofiche e nelle vaginosi", *Fitoterapia ed integratori in ostetricia e ginecologia,* Facchinetti F., Unfer V. CIC Edizioni Internazionali - Roma http://www.gruppocic.com/scheda_libri.php?INDICE=1&ID=7531-6.
19) **De Petrocellis L, Deva R, Mainieri F, Schaefer M, Bisogno T, Ciccoli R, Ligresti A, Hill K, Nigam S, Appendino G, Di Marzo V. (2009)** Chemical synthesis, pharmacological characterization, and possible formation in unicellular fungi of 3-hydroxy-anandamide, *J. Lip. Res.,* **50:** 658-666.

20) **Di Pierro F, Neri M, Bollero C. (2009)** Trattamento della psoriasi – Efficacia di un preparato multicomponente, *Cosmetic Technology,* **12(2):** 13-17.

21) **Artaria C, Maramaldi G, Bonfigli A, Rigano L, Appendino G. (2011)** Lifting properties of the alkylamide fraction from the fruit husks of *Zanthoxylum* Bungeanum, *Int. J. Cosmetic Science,* in press.

22) **Artaria C, Maramaldi G. (2010)** WO/2010/094477, "Cosmetic Wrinkel Treatment Method Based On *Zanthoxylum* Bungeanum Extract", 18 Feb. 2010.

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Embelin-A natural potential cosmetic agent

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Summary

Embelia ribes one of the Indian traditional medicinal plant, has been used as a cosmetic agent to cure skin disorders for centuries. *E.ribes* is used especially for dyeing hairs, good pimple remover, treating acne, treating carbuncle infections, treating vitiligo and leucoderma. *E. ribes* berries contain a quinone derivative embelin (2,5-dihydroxy -3-undecyl,1,4- benzoquinone), has a wide spectrum of biological activities, such as antioxidant, antitumor, anti-inflammatory and analgesic, antihelmintic, antifertility and antimicrobial. Quinone derivatives and the analogs; Ubiquinone (Coenzyme Q_{10}), Idebenone, Arbutin and Hydroquinone are well-known for cosmetic applications. In the present study, embelin from *E.ribes* berries of Indian origin was extracted and characterized by UV and FT-IR analyses.

Hemolytic, tyrosinase and DOPA auto-oxidation assays were also carried out. About 1.9± 0.1 gram of pure embelin was obtained from 100 gram of powdered berries *(E.ribes)*. The characteristics studies reveal the properties are on par with the standard embelin received from Sigma (USA). The half-maximal effective concentration (ED_{50}) of embelin to cause hemolysis was found as 109± 0.1 μg/ml. The tyrosinase inhibitory activity of embelin was nil and the DOPA auto-oxidation activity was observed up to 350 μg /ml concentration. Thus the embelin finds, potentially application in cosmetic industries.

Riassunto

L'Embelia ribes, una delle piante usate nella medicina tradizionale indiana, è stata utilizzata per secoli come agente cosmetico per curare alcune forme patologiche.

E. ribes è usata soprattutto per colorare i capelli, per eliminare le piccole pustole acneiche e per trattare la vitiligine e le leucodermie.

E. ribes contiene un derivato chinonico (2,5-diidrossi-3-undecil, 1, 4-benzochinone) che possiede un ampio spettro di attività biologiche

agendo come antiossidante, antitumorale, antiinfiammatorio, analgesico, antielmintico, antifertilità e antimicrobico.
Alcuni derivati chinonici quali l'ubichinone (coenzima Q_{10}) l'idebenone, l'arbutina e l'idrochinone sono ben conosciuti per il loro uso cosmetico.
Con il presente studio è stata estratta l'embelina dalla E. ribes caratterizzandola attraverso l'uso dell'UV, FT e IR. Ne è stata anche valutata l'attività emolitica e l'attività sulla tirosinasi e sulla catena DOPA.
Da 100 gr. di *E. ribes* in polvere è stato ottenuto circa 1.9 ± 0.1 gr. di embelina pura, che ha rivelato di possedere le stesse caratteristiche dell'analogo prodotto ricevuto dalla SIGMA (USA).
La concentrazione massima di embelina che provoca emolisi al 50% $8ED_{50}$) è di 109 ± 0.1 μg./ml.
L'attività inibitoria nei confronti della tirosinasi è risultata nulla, mentre l'attività autossidante nei confronti della DOPA è stata riscontrata pari a 350 μg/ml.
Per tali caratteristiche, l'embelina può trovare potenziali applicazioni d'uso nel comparto cosmetico.

INTRODUCTION

Beauty, the quality that gives pleasure to the senses, is perhaps the desire of every human being on earth especially in Asian. Some are born beautiful and some are in fact made beautiful. Aesthetic appearance has always been a matter of main importance. The word 'beauty' is not only related to women gender, as is often thought, but men also intend to use cosmetic products as daily basics. The knowledge of herbal cosmetics is booming now-a-days by both verbal transmitted folk information and newer information generated by modern scientific studies. Herbal products like extracts, oils and powders have been used in cosmetics as either active moieties or as excipients (as individually or in combination). Herbal extracts are primarily added as cosmetic ingredients due to several value added properties such as antioxidant, anti-inflammatory, anti-aging and anti-tyrosinase etc. Kole et al. (14) reported some Indian medicinal plants, which have been used in cosmetic preparations. And he added that *Embelia ribes* one among the plant has been used as a cosmetic agent to cure skin disorders for centuries. Samatha and Vasudevan (30) reported that aqueous extract of *E.ribes* on acid hydrolysis yield a colored product, which is used for dyeing cotton, nylon, silk and wool. And added *E.ribes* can be used as substitute for the synthetic red colors.

Samatha and Vasudevan (31) also reported embelic acid isolated from *E.ribes* for dyeing hair. However retention of the color is inferior to *Pterocarpus santalinus* extract studied. The uses of *E.ribes* have been mentioned in Ayurvedic texts. Susrut recommended *E.ribes* rasayana for longevity (anti-aging), while Vagbhata recommended *E.ribes* in treating leprosy, as an anthelmintic in ringworm and bulbous eruptions (33). *E.ribes* is one of active ingredient of TARIKA herbal pimple remover, product of Ayurlabs India, they conducted a clinical trial studies for the same and observed as good herbal pimple remover in all cases of acne patients (20).

Paranjpe and Kulkarni (21) reported one of Ayurvedic formulation namely; Sunder Vati showed a significant improvement in inflammatory and non-inflammatory lesions compared with baseline or placebo in Indian patients with *Acne vulgaris*. Anand Kumar and Sachidanand (2) conducted a clinical trial studies with new polyherbal formulations CLARINA cream and PURIM tablets. *E.ribes* is one of ingredients of CLARINA cream and thus reported that CLARINA cream along with PURIM tablets was useful in treating patients with various degrees of acne. Saikia et al. (28) reported *E.ribes* along with *Emblica officinalis, Piper longum and Terminalia belerica* all the four herbs are mixed equally and the crushed form dissolved in honey and applied for treating carbuncle infections. "PIGMENTO", an ayurvedic tablet marketed by Herbal Ayurvedic Remedies (www.herbal ayurvedic remedies.com) also contains *E. ribes* as one of ingredients, which is used for treating vitiligo and leucoderma.

The Government of India recently has set up a National Medicinal Plants Board (under the Ministry of Indian System of Medicine and Homeopathy) for over all development of medicinal plants and its knowledge. The Board has identified 32 prioritized medicinal plants, *E.ribes* is one of plant which has gained national importance owing to its therapeutically and commercial need especially of *E.ribes* berries (29). *E.ribes* is also one among the top 20 ayurvedic drugs of India as reported by Patwardhan et al. (23).

E. ribes berries contain a quinone derivative embelin, an alkaloid christembine, a volatile oil and vilangin. (26). Among them, embelin is considered one of the major bioactive constituents and marker compounds in *E. ribes* berries (4; 5; 9). Embelin (2, 5-dihydroxy -3-undecyl, 1, 4-

benzoquinone) has a wide spectrum of biological activities, including antioxidant (13), antitumor (10), anti-inflammatory & analgesic (8), antihelmintic, antifertility (15) and antimicrobial (9).

In the present study, we have extracted embelin from *E.ribes* (Indian origin) and examined the hemolytic potential of embelin (2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, 4-benzoquinone) in human erythrocytes. And also investigated the mushroom tyrosinase activity and DOPA autooxidation potential of embelin.

MATERIALS AND METHODS

Plant material

E.ribes berries were obtained from M/s Abirami Botanical Corporation, (Tuticorin, TamilNadu, India) and it was authenticated by Dr. Anandan, Research Officer, Anna Hospital, Chennai.

Extraction of the Embelin

Extraction of embelin was carried out according to Indian herbal pharmacopoeia (3). 100 g of the powdered berries of *E.ribes* was extracted with n-hexane using a soxhlet extractor for 6 hrs. The extract was then evaporated on rotator vapor and recrystalized using ethanol and chloroform and characterized using UV Visible and FT-IR analyses according to the standard methods.

UV-Visible spectrum was recorded using UV-2450, Shimadzu (Japan) in the wavelength range between 190-800 nm. FT-IR spectral measurements was made using Spectrum one (Perkin-Elmer Co., USA model).

Hemolytic activity

The hemolytic activity of plant materials or its preparation was determined using suspension of erythrocytes cells (RBC), mixed with equal volumes of a serial dilution of the plant material according to WHO guidelines (34).

Blood sample was aseptically collected in hepairnized sterile tube from healthy volunteer, after getting their consent for experiment. Blood samples were centrifuged at 10,000 rpm for 20 minutes at 4°C, to remove the cell debris. Resultant pellet was washed (3-4 times) repeatedly with phosphate buffer saline (PBS) pH 7.4 to obtain erythrocytes cells (RBC). This resultant erythrocyte cells (RBC) was suspended in phosphate buffer saline containing test solution at concentration of 20-125 µg /ml and was incubated at room temperature for 10 minutes in the dark. At end of incubation, tubes were centrifuged at 6000 rpm for 20 minutes at 4°C, in order to separate the intact cell and debris.

The amount of released hemoglobin (Hb) in the supernatant was measured spectrophotometrically at 540 nm. The half-maximal effective concentration (ED_{50}) of hemolysis was then calculated from the resulting dose response curve.

Tyrosinase activity

Tyrosinase activity was determined by Dopachrome method using L-tyrosine and mushroom tyrosinase as substrate and enzyme source respectively as reported earlier by Radhakrishnan et al. (25). In brief reaction mixture of control tube constitutes 235 µl of 3 mM L-tyrosine, 285 µl of 50 mM phosphate buffer (pH 6.8) and 180 µl of mushroom tyrosinase. In the case of experimental samples, buffer and test solution was adjusted accordingly. The assay mixture was incubated at 37°C, 10 minutes prior to enzyme addition and 20 minutes after enzyme addition. The pink color formed (Dopachrome) was measured spectrophotometrically at 475 nm. Kojic acid was used as reference.

DOPA auto-oxidation assay

DOPA auto-oxidation was determined by Dopachrome method using L-DOPA and Riboflavin as substrate and source of producing oxygen radical respectively as reported earlier by Radhakrishnan et al. (25). In brief reaction mixture of control tube constitutes 250 µl of 4 mM DOPA, 200 µl of 26 µM Riboflavin and 550 µl of 50 mM phosphate buffer (pH 7.5). In the case of experimental samples, buffer and test solution was adjusted accordingly. The assay mixture was irradiated under fluorescent lamp for 15 minutes. The pink color formed (Dopachrome) was measured spectrophotometrically at 475 nm. Kojic acid was used as reference.

RESULTS

About 1.9± 0.1 gram yield of pure embelin (figure.1) was obtained from 100 gram of powdered berries (*E. ribes*). In the UV spectrum, embelin exhibited λmax at 289 nm as shown in the figure.2; similarly IR spectrum exhibited various characteristics functional groups as shown in the table.1. Other instrumental analyses, viz., NMR, DSC, TGA and XRD, were carried out and the obtained results are compared with the standard embelin obtained from Sigma, USA (results are not shown) and found the characteristic features are on par with the standard embelin.The half-maximal effective concentration (ED_{50}) of embelin to cause hemolytic was found as 109± 0.1 mg/ml. Embelin does not have any action on reducing or inhibiting the Mushroom tyrosinase activity even at 125µg /ml, whereas the kojic acid (reference compound) showed half-maximal inhibitory concentration (IC_{50}) as 1.9± 0.1 µg /ml. Similarly the chosen test compound does not have any action on reducing or inhibiting the DOPA auto-oxidation reaction even at 350 µg /ml, whereas reference compound (kojic acid) showed half-maximal inhibitory concentration (IC_{50}) as 197± 0.2 µg /ml.

DISCUSSION

In the present study extraction of embelin from 100 gm of *E.ribes* using hexane provided 1.9± 0.1 gram of pure embelin, while Chitra et al. (9) reported 0.3 g yield of embelin. However, Chauhan et al. (5) and Madhavan et al. (18) reported 4.8 g and 3.8 g yield of embelin respectively. Followed by extraction the characterization studied of embelin revealed identical UV spectra peak similar to the results of Madhavan et al (18), Shelar et al. (32) and Babu Ganesan et al. (4). With regard to FT-IR analysis of embelin, the results are similar to the observation made by Madhavan et al. (18), Kumara Swamy et al. (16), Pathan & Bhandari (22) and Cherutoi et al. (6).

TABLE I

FT-IR analysis of embelin extracted from E.ribes.

S.No	Wave number(cm^{-1})	Assignment
01	3309	Hydroxyl group
02	2923	Alkane group
03	2854	Methylene group
04	1612	Carbonyl group
05	1326	Aromatic C-O streching
06	771	Benzene group

With reference to toxicity of embelin, Chen & Chen (7) reported that overdose of embelin can lead to renal toxicity. Pichaya and Warinthorn (24) reported embelin, showed high cytotoxicity against brine shrimp at LC_{50} 1.72 µg /ml, while Alluvri et al. (1) reported *E.ribes* berries, showed lethality against brine shrimp at LC_{50} 463 µg /ml after 24 hrs exposure. Haq (12) reported *E.ribes* causes visual defects leading to optic atrophy. The eye irritation and primary skin irritation studies of embelic acid conducted by Samatha & Vasudevan (31) revealed that embelic acid is non-irritant. In addition, no reports are available on the hemolytic activity of embelin. In the present study ED_{50} of embelin to cause hemolysis was found as 109± 0.1 mg/ml. However, Kuznetsova et al. (17) reported hemolytic activity for 2, 3-dimethoxy-5-methyl-6-polyprenyl-1, 4-benzoquinone (Ubiquinone Q_9) with concentration range of 30-50 µg /ml in mouse erythrocytes.

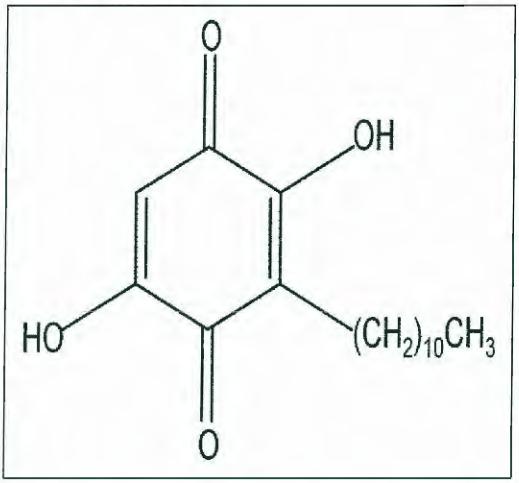

Fig. 1 *Represents the chemical structure of embelin (2, 5-dihydroxy -3-undecyl, 1, 4- benzoquinone).*

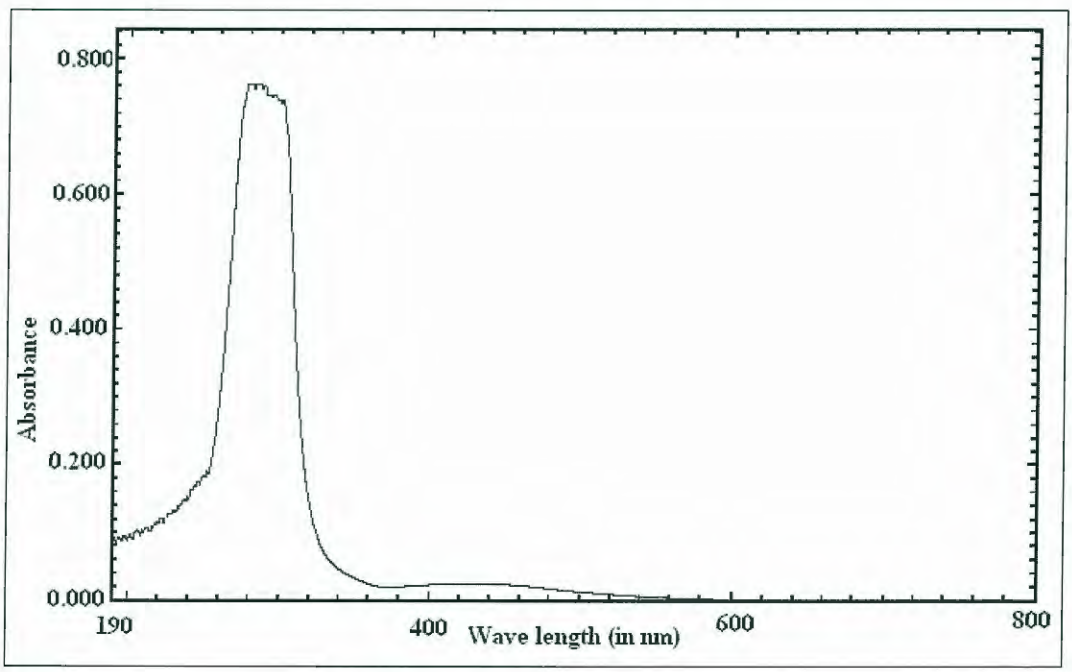

Fig. 2 *Represents the UV-Visible spectrum of embelin extracted from E.ribes.*

Zinkham and Oski (35) reported henna *(Lawsonia alba)* a cosmetic agent as potential cause of oxidative hemolytic and neonatal hyperbilirubinemia. While Raupp et al. (27) also reported henna (L.alba) causes life threatening hemolytic particularly in individuals with a genetic deficiency in erythrocytic glucose-6-phosphate dehydrogenase activity. McMillan et al. (19) observed no hemolytic activity for lawsone (2-hydroxy-1, 4-naphthoquinone) an active ingredient of henna *(L.alba)* and also for THN (1, 2, 4-Trihydroxynaphthalene) even at high concentration (>3 mM). Ediriweera et al. (11) reported *E.ribes* as one of the ingredient of Kaishor guggulu ayurvedic preparation used for treating shvitra (Vitiligo), similarly results of present study emphasize, embelin does not inhibit the tyrosinase activity and DOPA auto-oxidation activity and suggests its potential use as a cosmetic agent.

CONCLUSION

Thus the embelin finds, potentially application in pharmaceutical and cosmetic industries.

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References

1) **Alluri V, Krishnaraju T, Rao VN, Dodda S, Mulabagal V, Hsin-Sheng T, Gottumukkala VS. (2006)** Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (Brine shrimp test). *Int. J. Appl. Sci. Eng.*, **4:** 115-125.
2) **Anand Kumar BH, Sachidanand YN. (2001)** Treatment of *Acne vulgaris* with new polyherbal formulations, Clarina cream and Purim tablets. *Indian J. Dermatol.*, **46:** 138-141.
3) **Anonymous (2002)** In: Indian Herbal Pharmacopeia Revised edn. Indian Drug Manufacturer's Association, Mumbai, p.500 – 501.
4) **Babu Ganesan, Perumal P, Vijaya BM, Srujana DG, Surya Rao S, Latha ST. (2010)** Optimization of extraction conditions for embelin in *Embelia ribes* by UV spectrophotometry. *Arch. Apll. Sci. Res.*, **2:** 49-53.
5) **Chauhan SK, Singh BP, Agrawal S. (1999)** Determination of embelin from *Embelia ribes* by high performance thin layer chromatography. *Indian Drugs*, 36: 41–43.
6) **Cherutoi JK, Cheruiyot LL, Kiprono CP.(2005)** Synthesis and characterization of Zinc (II) and Copper(II) complexes of embelin. *Bull. Chem. Soc. Ethiop.*, **19:** 295-299.
7) **Chen JK, Chen TT. (2004)** In: Chinese medical herbology and pharmacology. 2nd ed. California: Art of Medicine Press.
8) **Chitra M, Sukumar E, Suja V, Devi CS. (1994)** Antitumor, anti-inflammatory and analgesic property of embelin, a plant product. *Chemother.*, **40:** 109-113.
9) **Chitra M, Devi CS, Sukumar E. (2003)** Antibacterial activity of embelin. *Fitoterapia*, **74:** 401-403.
10) **Chitra M, Devi CS, Sukumar E. (2004)** Effect of embelin on carbohydrate moieties of glycoprotein in tumor bearing rats. *J. Nat Rem.*, **4:** 77-80.
11) **Ediriweera ERHSS, Kalawana OTMRKSB, Karunarathna N, Nanayakkara NGAS. (2009)** Clinical study on efficacy of the traditional Sri Lankan oil 'The Kakodumbaradi Taila' with selected ayurvedic preparations on Shvitra (Vitiligo) *Ayu.*, **30:** 225-231.
12) **Haq I. (2004)** Safety of medicinal plants. *Pak. J. Med. Res.*, **43:** 203-210.
13) **Joshi R, Kamat JP, Mukherjee T. (2007)** Free radical scavenging reactions and antioxidant activity of embelin: Biochemical and pulse radiolytic studies. *Chemico-Biol. Inter.*, **167:** 125-134.
14) **Kole PL, Jadhav HR, Thakurdesai P, Nagappa AN. (2005)** Cosmetics potential of herbal extracts. *Nat. Prod. Rad.*, **4:** 315-321.
15) **Krishnaswamy M, Purushothaman KK. (1980)** Antifertility properties of *Embelia* ribes: (embelin). *Indian J. Exp. Biol.*, **18:** 1359–1360.
16) **Kumara Swamy HM, Krishna V, Shankarmurthy K, Abdul Rahimam B, Mankani KL, Mahadevan KM, Harish BG, Raja Naika H. (2007)** Wound healing activity of embelin isolated from the ethanol extract of leaves of *Embelia ribes* Burm. *J. Ethnopharmacol.*, **109:** 529-534.
17) **Kuznetsova TA, Dmitrenok AS, Sobolevskaya MP, Shevchenko LS, Mikhailov VV. (2002)** Ubiquinone Q_9 from a marine isolate of an actinobacterium *Nocardia* sp. *Russ. Chem. Bull.*, (Int.Ed.), **51:** 1951-1953.
18) **Madhavan SN, Arimhoor R, Arumughan C. (2010)** RP-HPLC-DAD method for the estimation of embelin as marker in *Embelia ribes* and its polyherbal formulations. *Biomed. Chromatogr.*, **25:** 600-605.

19) **McMillan DC, Sarvate SD, Oatis JE Jr, Jollow DJ. (2004)** Role of oxidant stress in lawsone-induced hemolytic anemia. *Toxicol. Sci.*, **82:** 647-655.
20) **Mishra B, Mohanpatra A, Krushna B.(2008)** Clinical trial done for TARIKA 100 % herbal pimple remover. (www.ayurlabs.com).
21) **Paranjpe P, Kulkarni PH.(1995)** Comparative efficacy of four Ayurvedic formulations in the treatment of *acne vulgaris*: a double-blind randomised placebo-controlled clinical evaluation. *J. Ethnopharmocol.*, **49:** 127-132.
22) **Pathan RA, Bhandari U. (2011)** Preparation and characterization of embelin-phosopholipid complex as effective drug delivery tool. *J. Incl. Phenom. Macrocycl. Chem.*, **69:** 139-147.
23) **Patwardhan B, Vaidya ADB, Chorghade M. (2004)** Ayurveda and natural products drug discovery. *Curr. Sci.*, **86:** 789-799.
24) **Pichaya P, Warinthorn C. (2002)** Bioactive phenolic lipids from the fruits of *Ardisia colorata Roxb. (www.thai.com)*.
25) **Radbakrishnan N, Gnanamani A, Sadulla S. (2005)** Effect of licrorice *(Glycyhrriza glabra Linn)* a skin-whitening agent on black molly *(Poecilia latipinnaa)*. *J. Appl. Cosmetol.*, **23:** 149-158.
26) **Rao CHB, Venkateswarlu V. (1961)** Chemical examination of *Embelia ribes*. I. Isolation of a new constituent, 'Vilangin,'its constitution and synthesis. *J. Org. Chem.*, **26:** 4529–4532.
27) **Raupp P, Hassan JA, Varughese M, Kristiansson B. (2001)** Henna causes life threatening haemolysis in glucose-6-phosphate dehydrogenase deficiency. *Arch. Dis. Child.*, **85:** 411–412.
28) **Saikia AP, Ryakala VK, Sharma PS, Goswami P, Bora U. (2006)** Ethnobotany of medicinal plants used by Assamese people for various skin ailments and cosmetics. *J. Ethnopharmocol.*, **106:** 149-157.
29) **Sahoo S. (2004)** CSIR technology on aromatic medicinal and spice plant for rural development. *J. Rural Tech.*, **1:** 91-95.
30) **Samatha S, Vasudevan TN. (1996a)** *Embelia ribes* Burm. as a source of red colorant, *J. Sci. Ind. Res.*, **55:** 888-889.
31) **Samatha S, Vasudevan TN. (1996b)** Natural hair dyes. *J. Sci. Ind. Res.*, **55:** 885-887.
32) **Shelar R, Maurya C, Tekale P, Katkar K, Naik V, Suthar A, Chauhan VS. (2009)** Embelin-An HPLC method for quantitative estimation in *Embelia ribes* Burm. F. *Int. J. Pharm. Clin. Res.*, **1:** 146-149.
33) **Singh G, Ahmad M, Nazim M.(2008)** A bibliometric study of *Embelia ribes*. Lib. Rev., **57:** 289-297.
34) **WHO (1998)** In: Quality control methods for medicinal plant materials. World Health Organization (WHO), Geneva. p. 41-43.
35) **Zinkham WH, Oski FA. (1996)** Henna: a potential cause of oxidative hemolysis and neonatal hyperbilirubinemia. *Pediatrics*, **97:** 707-709.

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