A large number of natural compounds may often represent the solution for a wide number of problems in human health. In fact, their pharmaceutical activities detain a large range of action, i.e. from the active ingredients against the most serious diseases to the molecules able to reduce the slight blemish (1).

Plant extracts have been used for healing purposes long before recorded history, as demonstrated by ancient Chinese and Egyptian papyrus writings describing medicinal plant uses. Several African populations used plants in their curative rituals, while others, such as indigenous populations of North America, developed traditional medical systems in which herbal therapies were used systematically (2,3). At the present time, people in different parts of the globe use plant seeds, berries, roots, leaves, bark, or flowers for the same purposes.

In the early 19th century, when methods of chemical analysis became available, scientists began to extract and to study several active ingredients from plants. A wide number of active products deriving from plant extracts have been included into nutritional supplements and used to correct imbalances, resolve patterns of dysfunction and treat the cause of complaint. Several molecules have been extracted and used to treat many human pathologies such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue and irritable bowel syndrome (4).

The nutritional supplements have been termed as Nutraceuticals, as they are defined as “a category of substances that may provide medical or health benefits, including prevention and treatment of disease” (5). As the consumption of nutritional supplements and the improvement of lifestyle require time to show their effects, nowadays, consumers express an increasing request of immediate benefits complementary to Nutraceuticals. To support this need of new molecules and systems to obtain the desired short-term benefits, the cosmetic industry has recently developed combinations of cosmetic formulations enriched with pharmaceutical compounds, thus new categories of products have been developed, denoted Cosmeceuticals (6).

The rising of this innovative perspective in cosmetics has directed the research activities of Pierre Chimica Laboratories to study and produce cosmetic products...
characterized by active natural molecules used in their native form. Although a common approach of the cosmetic industry is to reproduce chemical versions of natural compounds, transforming raw extracts into synthetic pharmaceuticals, the new research approach proposed in the present paper is based on the idea that the cosmetics have to be produced using natural compounds.

Our research was directed to: i) select active natural ingredients able to offer natural solutions in cosmetic approaches; ii) develop new production technology allowing to obtain natural compounds exhibiting the smallest structural and compositional changes in the extracted molecules; iii) develop new administering approaches and new formulas able to stabilize the active ingredients.

The above research has been focused in developing a gel-emulsion that combines the emollient and nutritive properties of vegetable oils (derived from almond, olive and jojoba) with the antioxidative activity of anthocyanins from red fruits extracts, such as Vitis vinifera L., Ruscus aculeatus L., Smilax aspera L., Laurus nobilis L.

Many plants, such as Aesculus hippocastanum, Centella asiatica, Ginkgo biloba, Ruscus aculeatus, Vitis vinifera, are known because of their flebotonic activity, and many medicinal remedies used in the treatment and prophylaxis of vein disorders are derived from them (7,8).

The occurrence of leg vein alterations and varicose veins is an annoying and recurring phenomenon, directly related to the sedentary way of life. These ailments are now becoming a “significant public health problem" (9) because of their incidence and the collateral effects, as demonstrated by different studies reporting that 60% of European citizens exhibit leg vein abnormalities (10), and the percentage of adult population affected by vein disorders is approximately 40% in the USA (11).

Although such disorders are not life-threatening, they usually cause discomfort and have an adverse effect on the quality of life. These pathologies are caused by the compromising of leg veins walls and/or valves and their risk factors can be resumed in: age, sex, and lifestyle, such as the unbalanced diet and the lack of physical exercise.

The remedies to treat or prevent vein disorders consist also of cosmetic preparations based on antioxidant molecules. The antioxidants can prevent lipid peroxidation by several mechanisms, including scavenging lipid peroxidation-initiating radicals, binding metal ions, scavenging lipid peroxyl radicals and inhibiting enzymatic systems responsible for free radical production (12). Anthocyanins showed the ability to protect both large and small blood vessels from oxidative damage (13-16), thus permitting to be included into new cosmeceutical formulations for the treatment of achy and heavy legs.

The traditional extracts of red fruits, particularly rich in anthocyanins, are available as dry extracts obtained by using chemical solvents, such as methanol, chloroform, dichloromethane, etc. But the extracts are absorbed by the body, being toxic even in ppm concentrations.

In collaboration with the research group of Prof. Giuseppe Vasapollo (Università degli Studi di Lecce, Dipartimento di Ingegneria dell’Innovazione), many typical plants growing in the Mediterranean area were investigated as possible new sources of anthocyanins.

The different plant species considered during our research projects are Vitis vinifera var. Malvasia nigra, Rhamnus alaternus (17), Laurus nobilis, Ruscus aculeatus (18), and Smilax aspera.

In order to produce an extract of antioxidant compounds preserving their original properties, Pierre Chimica Laboratories developed an innovative process of extraction, concentration, and purification to obtain anthocyanins from red fruits, using supercritical carbon dioxide (SC-CO₂) as solvent (Figure 1).

The anthocyanin molecule is normally constituted by two different portions, respectively named aglycon and sugar (Table I). The combination of a different aglycon with a different sugar generates different anthocyanin species. Each plant contains a specific anthocyanin profile, consisting of a mixture of peculiar combination of different anthocyanin molecules.

The extraction process of natural anthocyanins with SC-CO₂ is shown in the flow diagram in Figure 2. The first step in the extraction process consisted of crushing the berries and stirring them in a hydroalcoholic solution in the absence of light. The obtained slurry was then filtered and directly injected into the SC-CO₂ flow.

After the contact between SC-CO₂ and the hydroalcoholic mixture, a phase separation occurred: one phase contains CO₂ and all the components soluble in CO₂, i.e. ethanol and water in traces; the second phase contains all the components insoluble in CO₂, i.e. water and anthocyanins.
The SC-CO₂ soluble substances are a range of chemicals with different molecular weights and polarity. They exhibit a solubility pattern inversely proportional to their molecular weights and to their polarity at any given temperature and pressure. Comparing chemicals with the same polarity, lower molecular weight components, such as ethanol, have higher solubility in CO₂, while higher molecular weight components have a lower solubility and consequently lower extraction rate (19). The presence of different organic substituent groups, affecting polarity, also affects the solubility.

The phase containing water and anthocyanins was finally collected as Fraction 1 (Figure 2) and used as aqueous extract for high quality cosmeceutical emulsion preparation.

The advantages deriving from the use of CO₂ supercritical fluid extraction appear to be numerous when this new approach is compared with traditional methods, which involve chemical solvent extraction. The very high selectivity of CO₂ avoid the dissolution of polar substances along with lipophilic substances, the absence of heat treatment (the operating extraction temperature in the SC-CO₂ process is less than 40-50°C) for solvent removal eliminates the polar polymers formation. No extra unit operations for solvent removal are needed and yield of target compounds is very high.

This novel extraction procedure preserved the properties of natural anthocyanins, thus allowing the preparation to be considered as a natural extract.

The plant extracts used in cosmetic applications are commercially dispensed in several different forms such as oils, liquid and dry extracts (powders).

With the aim of introducing an active extract of anthocyanins in a new cosmeceutical formula, we planned to produce an emulsion as topical delivery system. In fact, this form is the most suitable for preserving the activity of the ingredients and the most diffused formulation for topic administering, also for its high compliance by the consumers.

In collaboration with the research group of prof. Lorenzo Vasanelli ("Università degli Studi di Lecce, Dipartimento di Ingegneria dell’Innovazione"), Pierre Chimica Laboratories assayed the aqueous extracts obtained by SC-CO₂ system in comparison with the commercially available dry extracts. The results of these tests, obtained using the ORAC method (13, 14), are shown in Table II.

These tests revealed an antioxidative activity of the SC-CO₂ extracts two-fold higher than commercial extracts, thus encouraging Pierre Chimica Laboratories to promote the use of SC-CO₂ extracts from Vitis vinifera var. Malvasia Nigra as an active principle for a cosmeceutical formula for the treatment of achy and heavy legs.

A new gel-emulsion formula, with the high emollient properties from the algae, the nutritive characteristics of the vegetable oils, and the refreshing power of menthol has been elaborated. Moreover, this emulsion showed a pleasant skin feel, a good emollient effect on the skin and a rapid absorption. The formulation has been separately modified by addition of the following extracts: i) aqueous extract of Vitis vinifera var. Malvasia nigra, purified and concentrated by SC-CO₂, and ii) commercial dry extract of Vaccinium myrtillus, as control.

The above new formulations were validated on a panel of 30 women (30 to 50 year-old) affected by achy and heavy legs, to whom the emulsions were applied twice a day for fifteen days (Table III).

The extract of Ruscus aculeatus was not included in the formulation utilized for the panel test, due to its lower antioxidative activity than the Vitis vinifera var. Malvasia nigra one.

The results obtained testing the formulations on the customer panel appeared to be highly positive. In fact, all the treated subjects felt the following short-term benefits: reduced swelling of the ankles, lower tightness of the calves, reduction of the leg tiredness and increased cutaneous microcirculation ("fresh heat" sensation). Investigations are now in progress in order to develop a potential line of products with treatment personalization, by optimizing specific formulations with different percentages of each extract.

### CONCLUSIONS

In the next future, the industrial goal of Pierre Chimica Laboratories will be to optimize the extraction and purification of natural anthocyanin process in order to obtain a standardized production of natural extracts.

As experimentally demonstrated, the extracts are free of undesirable components, such as anthocyanin decomposition or hydrolysis products. In fact, the supercritical extraction process allows to avoid any temperature stress (generally responsible of distortions or alterations in the nature of the delicate plant constituents) and ensures the biologic stability of the active ingredients, with the added benefit of an extract free of any chemical solvent residue.

The extracts containing anthocyanins, obtained by our

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<th>Table II – Antioxidative activity of different extracts, expressed in ORAC/ml</th>
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<tr>
<td><strong>Extract</strong></td>
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<tr>
<td>Vitis vinifera var. Malvasia nigra SC-CO₂</td>
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<tr>
<td>Ruscus aculeatus SC-CO₂</td>
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<tr>
<td>Vaccinium myrtillus dry extract (Commercial)</td>
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<tr>
<td>Pharnax niveus</td>
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<td>Laurus nobilis</td>
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<td>Simix aspora</td>
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<th>Table III – Results of the tests on the panel of 30 women</th>
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<td><strong>Comment</strong></td>
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<tr>
<td>The cream has a pleasant skin feel</td>
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<td>The cream is rapidly absorbed</td>
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<td>The cream has an intense moisturizing effect</td>
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<td>The cream is irritant</td>
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<td>The cream has a relaxing effect:</td>
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<td>Soft</td>
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<td>Medium</td>
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<td>Strong</td>
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<tr>
<td>The cream has an action against swollen legs:</td>
</tr>
<tr>
<td>Soft</td>
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innovative process involving SC-CO₂ have demonstrated higher activity than the commercial dry ones. The peculiar properties of these extracts encouraged their potential inclusion into cosmeceutical formulas in the treatment of swollen and heavy legs.

Moreover, the gel emulsion developed by Pierre Chimica Laboratories is a very light cream, free of any oily effect, combining the rapid absorption by the skin and the prolonged stimulating and refreshing sensation. The consumer favorable opinion and the remarkable reported benefits in terms of reduction of pain and sense of achy legs, are considered interesting and important results for the proposed formulation.

ACKNOWLEDGEMENT
The Authors gratefully thanks Dr. Francesco Grieco, Dr. Gianluca Bleve and Dr. Paride Papadia for critical reading.

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