INTRODUCTION

Nowadays the use of vegetal derivatives as raw material for cosmetics, is always increasing and recently some divulgative articles describe several functional characteristics of different vegetal by-products.
For instance, the extracts of Rosemary have been introduced as a "tonic for cutaneous tissues", as a "balancing substance" and in particular as a "stimulant" (1).
In this study we will analyse the acquired knowledge of such extracts, mainly *Rosmarinus officinalis* L. as seen through scientific literature.

EXTRACT FROM *ROSMARINUS OFFICINALIS* L.

Rosmarinus officinalis L. is a very common evergreen plant present in the Mediterranean area.
Due to its content of essential oil and also due to the presence of phenolic compounds, which includes interesting molecules with possible antioxidant properties, this species is used to a great extent in all foodstuff and, recently, also in the herbal and cosmetic sectors (2).

THE NATURE OF ESSENTIAL OILS

The most characteristic and particular compounds in the essential oil of the major part of the plants are terpens and terpenoids. They show a carbon skeleton made up of multiples of C_5 branched isopren unit, which grows from monomer to highly polymerised rubber (molecular weight 10^6). The low boiling fragrant fraction includes terpens (C_{10}) and sesquiterpens (C_{15}) (3).
Usually the term "terpens" indicates the hydrocarbonic derivates, and "terpenoids" the oxygenated ones (alcohols, esters, aldehyds and ketons).

THE ORIGIN OF ESSENTIAL OILS

These group of natural products are synthesised by well known biochemical pathways, which begin with the condensation of three acetylCoA in two steps to form HMGCoA, which is reduced to mevalonic acid, the committed precursor of all isoprenoid derivatives. Phosphorilation and decarboxylation reactions with the elimination of the C_3 oxygen fuction yield isopentenyl pyrophosphate, the precursor of both geranyl pyrophosphate and farnesyl phyrophosphate, the bulk respectively of monoterpens and sesquiterpens (4).
More of fifty vegetable species contain essential oils, from the *Pinaceae* to the *Orchidaceae*, from the *Ranuncolaceae* and *Asteraceae* to, obviously, the *Lamiaceae* (5).
Surprisingly, considering the synthetic capacity of higher plants and the evolutionary time scale for development, the compounds extracted are quite similar to each other, even if they derive from genetically distant families characterised by different biosynthetical pathhways (6).
An emblematic case is the presence of terpens in *Rosmarinus officinalis* L. such as:

- 1,8-cineole (also known as eucalyptol), which is mainly present in the essence of *Eucalyptus globulus* Labill.fam. *Myrtaceae*
- camphor, a compound contained in significant quantities in *Cinnamomum camphora* Nees & Eberm. fam. of *Lauraceae*
- linalool, present as acetate in *Lavandula officinalis* Chaix. fam. *Lamiaceae* and in *Citrus bergamium* Risso, fam. Rutaceae
- limonene, commonly found in genus Citrus, fam. Rutaceae.

THE ROSEMARY ESSENCE

The rosemary essence is a complex mixture of terpens and terpenoids whose composition has been intensively studied.
Although it has been noticed that usually there

is an almost identical percentage of a-pinene, 1-8 cineole and camphor (7), some of the recent works underline that other compounds have been found in variable amounts. The complete absence of 1-8 cineole was recently reported in an Egyptian sample (8).
The possible conclusion is that there are different chemotypes in the same species; i.e. characteristic of the rosemary are 1-8 cineole chemotypes, the verbenon one, and those with a prevailing level of myrcene (8, 9).
When the composition of essential oil was analysed in different varieties with blue and white flowers, it was observed that from the distillation of the blue flower plants a lower amount of a-pinene and 1-8 cineole (-30%) was present, while a double quantity of borneol and borneol acetate and a larger amount of verbenon are found as compared to the extract from white flower type.
These differences are also found in the scent of the essential oil: in the blue plants the scent is a typical one, strong, pleasant, balsamic and sweet, whereas in the white flower plants it is less strong, herbaceous, sour and drier (10).
It is also important to keep in mind factors such as the season and the stage of vegetal life during the harvest, which have been demonstrated to be significant factors for the differences found in the composition of the obtained oils (11). Furthermore it must be pointed out that wild plants are characterised by less amount of essence compared to the cultivated plants, both "erect" and "creeping" varieties (12).
Finally it is worthy note that essential oils are complex natural products which can not be completely described by their major components.
In fact for the essential oil of *Rosmarinus officinalis* L. described in the Italian Official Pharmacopea (FU IX) it is highly emphasised that: "oil from different batches must not be mixed" (13).

THE PHENOLIC FRACTION

In the early 50's it was shown that a rosemary leaf extract showed an intense antioxidant activity; commercial products were soon used in the food industry for that purpose (14, 15).
The antioxidant activity is due to some phenolic compounds that have been studied in details: carnosol, carnosic acid, rosmaridiphenol, rosmanol, isorosmanol, epirosmanol, rosmariquinone (alias miltirone), ursolic acid, betulinic acid , ferulic acid, caffeic acid, luteolin glucuronide, hesperidin and genkwanin were found to be present, even in different amounts (12, 16, 17, 18).
Some considerations about the methods used for the extraction of these compounds can elucidate the problems that occurred during these studies. In plants phenols exist in different forms: as free molecules, as glycoside bound phenols and as phenols trapped in lignified water-insoluble structures. Different solvents can extract only a part of them according their polarity and there are differences in the extract due to the different pressure exerted (12).
Furthermore, under different cultivar conditions we have the same variations as seen in essential oils. Due to this very complex pattern careful studies for a correct identification of the active principles and on the synergism between the different molecules are needed.

ANTIOXIDANT ACTIVITY

The free radical scavenging activity of phenolic compounds from rosemary was measured by spin-trap technique: the effect was lower than vitamin C but higher than vitamin E (19). When SOD-like activity was measured good results were also obtained (20).
Some substances extracted from *Rosmarinus officinalis* L. showed stronger and more thermostable properties to inhibiting the oxidation of vegetable and animal fats as compared to BHT and BHA (21).

In another study a commercial extract inhibited peroxidation of phospholipid liposomes (22).
At the same time antimutagenic properties were also measured; carnosic acid was identified as the most powerful agent (23).
TPA is a molecule involved in PIP_2 degradation and arachidonic acid release; both events are related to PCK activation and are supposed to have prooncogenic properties. Other effects of those molecules are to produce in mice oedema and ornithine decarboxylase (ODC) increase. In this model a methanol extract of rosemary topically used was capable to significantly reduce all the above mentioned phenomena. (24).
Phenolic diterpens of rosemary were shown to protect biological systems as mitochondria, microsomes and red blood cell membrane against oxidative stress. As well known, membrane lipids are particularly susceptible to oxidation, not only due to their high polyunsaturated fatty acids content, but also to their association in cell membrane with enzymatic and not enzymatic systems capable to generate free radical species (25).
In this context rosemary extract and carnosol stimulated the activity of rat liver detoxifying enzymes gluthatione-s-transferase and quinone reductase (26).
Finally carnosol inhibited the nitric oxide (NO) production induced by cytokines during inflammation (27).
In front of these evidences some investigations showed that these compounds can act as prooxidative substances promoting DNA damage in the copper-phenantroline and in the bleomicin-iron assays (22, 28).

ANTIMICROBIAL ACTIVITY

An alcoholic extract of *Rosmarinus officinalis* L. has been used in order to eliminate Salmonella from foodstuffs (29), and the essential oil was proved to be active in vitro against *Candida albicans* (30).
A further study demonstrated that the oil from Egyptian rosemary plays a very active role in the prevention of growth of *Criptococcus neoformans* and *Mycobacterium intracellulare* (8)
Surprisingly it was also discovered that the same oil showed a vermicidal activity and moreover in another study the carnosol was proved to be a protease inhibitor of HIV1, even with weak but definite anti HIV1 activity (22). In view of this therapeutical potential new studies must be done to demonstrate definitely these properties.
However the essential oils are traditionally recognised for their antibacterial and antimycotic actions, even if devoid of high therapeutical activities.

AROMA-CHOLOGY

This neologism has been create to indicate the relationship between scents and psychology (31).
There are many mechanisms with which scents can exert effects on the human psyche and there are several temptative studies to provide solid scientific evidence to this effort.
One worth mentioning is the experiment in which the inhaling of essential oils showed a distinct increase in the quantity of human secretory immunoglobulins s-IgA (33); the rosemary oil proved to have a stimulating effect in another research using the CNV-technique, an experiment which measures specific fluctuations of the human EEG (34).
In the mouse another effect tested was the correlation between blood levels of 1,8-cineole and an increase in locomotory activity after inhalation of rosemary oil (35).
However many authors are doubtful towards testing the scents effects on humans, since further implications such as cultural, semantic and psychological factors are involved. The variability of the different results and the unreliable dose-effects obtained, also in the absence of a suita-

ble model, pose the question of how these tests could be applied to man as in many cases was made with more defined therapeutical molecules and drugs (36).

TOXICOLOGY

Even if a commonly herb used in foodstuffs such as rosemary could appear to be harmless, we cannot disregard the risks factors of the toxicology of its extracts, that could produce direct and indirect forms of toxicity.
For example one of the indirect toxicities worth mentioning is the presence of mercury in rosemary grown in the area of Monte Amiata (Grosseto, Italy) (37).
One commonly used model to assess drug toxicity is to investigate the induction of the enzymes involved in P450 system: when the components of some essential oil like 1,8-cineole, borneol and cadinene were assayed it was clearly demonstrated that they can induce some of the enzyme activities (38), even through inhalation (16), like drugs which for this reason could lead important toxicity influencing the metabolism of other molecules (39).
The 1,8-cineole and the limonene can alter a lamellar matrix model of intercellular lipids of stratum corneum. Results of differential calorimetric analysis denote a transition from the lamellar mesomorphic structure to an isotropic liquid phase (40): the resulting increase of the permeation through preferential channels could enhance penetration of undesirable substances.
As far as direct toxicity is concerned, we have had only one specific report on professional asthma due to rosemary in foodstuffs industry personnel (43); an acqueous extract (44) and by some essential components such as α-terpinene, myrcene, sabinyl acetate it was also reported (47). However, as before mentioned, even though the last mentioned components are present in very small amounts in the essential oil, variables above mentioned (cultivar, extraction methodology, used subspecies, climate and last but not least cultivation and harvest condition) can easily modify the quality and the quantities of the essential oil components.
The Registry of Toxic Effects of Chemical Substances published by the US Public Health Dept. indicates the rosemary oil as moderately irritating for skin and eyes (36). A more recent monograph published by Italian Istituto Superiore di Sanità, the institute with technical and consultant advisory faculties of the Ministry of Health, proposed that further toxicological data are required to define the use of rosemary extracts (16).

CONCLUSIONS

Although vegetable extracts are considered safe due to their traditional use in foodstuffs, we must not overlook studies of their mechanism of action, of the possible side effects and of the potential toxicity.
According to some authors the culinary use of these herbs is sufficient to guarantee their safety; but this it is not the case of cosmetic use; in fact these ingredients can yield a differently higher level of concentration on topical products mainly through the application of "stay-on" cosmetics, which remain on the skin for a certain period and are often and repeatedly used. It must be also pointed out that the properties in topic products are obtained with relatively high concentration; in fact many of these products contain a quantity like 1% to 5% of essential oil or even 0,1% of antioxidant extracts.
Even if the prediction of absorption of small lipophilic molecules such as fragrances is possible through mathematical formulas like the Potts-Guy equation (48), it is not so easy the evaluation of the absorption of complex mixtures, which can alter the stratum corneum lipids, with an increase of permeability.
Furthermore the composition of vegetal raw material significantly changes due to many dif-

ferent variables giving serious problems when the technological requirement is costancy for the quality and composition of the different products that are to be used.

The antioxidant and antibacterial activities and the psychological impact to the fragrance obtained in some experimental studies could lead to important uses for rosemary in cosmetics. However its activity on the skin that has been reported lacks sufficient scientific proofs.

In fact tests on human skin with Colorimeter, Laser Doppler, TEWL, Sebumeter, and Corneometer of cosmetic preparation containing some essential oils are reported to show very small effects and in most cases statistically not different from the ones produced by the cosmetic vehicle alone (49). These studies are essential when therapeutical effects on skin biology are claimed on product labelling.

In any case products obtained from nature are somehow part of the echosystem and terpenoids with low molecular weight appear to be suitable for the above mentioned cosmetic purposes.

All the vegetal derivatives have very well defined properties when present in their environment (for instance in the physiology of the plant where they are extracted from). In this sense the acute formulator may obtain predictive information on the possible potential use not only in terms of efficacy, but also in terms of safety.

The volatility of low molecular weight terpenoids provides their mobility, imparting the ability to influence another organism at some distance from the source plant. Complexity of composition and stereochemistry of the constituents confers the capability to communicate a selective biological message of attraction (for pollination and the spreading of seeds) or repulsion (from insects, herbivores, etc.). The lipophilic nature allows for some persistence in the largely aqueous biosphere, while unusual structural features, such as cyclopropyl and furan groups, promote a degree of biological stability, with possible toxic consequences for other life forms.

Having analysed all the above mentioned factors, it is easier for us to understand better the main problems connected to particular raw materials such as those extracted from essence plants.

We must be rational when we use complex vegetable extracts; it is only through this rationality that we can transform the feeling that drive us towards a "natural" choice in a very important factor in the cosmetics world: the capability to rise up emotions.

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