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# Guidelines for the Development of Herbal-Based Sunscreen

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Additional information is available at the end of the chapter

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## Abstract

Sun protection is a complex topic, which involves various classes of compounds. The photoprotective effectiveness of a sunscreen involves many biological activities, such as ultraviolet (UV) radiation filter properties and antioxidant, anti-inflammatory, and antimutagenic effects. Formulation strategy is also a key factor. Several studies have examined the role of natural molecules as photoprotective compounds, and a considerable number of commercially available sunscreens contain herbal extracts but not as sunfilters. Indeed, the process of evaluation of UV-filtering and photoprotective activity of herbal compounds presents certain specific difficulties and needs in vitro and in vivo studies. Nowadays, no natural compound or vegetal extract has been approved by any country as official UV filter for sunscreen. With these premises, the aim of this chapter is to define a set of tests, which can help to evaluate the efficacy of an herbal extract in the field of sun protection; in other words, we propose a rational approach to the discovery of natural UV-filtering extract and molecules. The following electronic databases have been used as a source of information: SciFinder, PubMed, Google Scholar, ISI-Web of Science, and Scopus.

**Keywords:** natural sunscreen, rational development, sustainable resources, SPF (sun protection factor), skin, cosmetic

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## 1. Introduction

The use of herbal extract and natural molecules in the field of solar protection represents a new trend in the cosmetic industry; in fact, over the last few years, a significant increase of the usage of herbal extracts has been registered given the growing interest of the customer for

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“green” and “natural” ingredients in the finished product. A large number of studies appearing in scientific literature demonstrate the photoprotective activity of natural products due to UV-filtering activity, antioxidant activity, and DNA-protecting effects [1–3]. Despite these findings, studies regarding photoprotection activity have been developed with a wide range of different methods and different strategies of investigation, thus making difficult to understand both the actual and the claimed potential of the activity. The main cause of this situation is the lack of repeatability of the *in vitro* SPF tests available nowadays.

In the USA, sunscreen is classified as an OTC (over-the-counter) product. In the European Union (EU) countries, it is listed as cosmetics, and its production must follow the EC Regulation 1223/2009 of the European Parliament on Cosmetic Products. Regarding the SPF determination, the ISO 2444:2010 is nowadays regarded as the reference method; this method involves an *in vivo* procedure carried out on human volunteers. Due to ethical problems, the *in vivo* UVAPF determination by the ISO 24442:2011 has been recently substituted by the *in vitro* ISO 24443:2012 method.

The “gold standard” for the SPF determination nowadays is provided by the ISO 2444:2010 *in vivo* test. It is not applicable in the initial screening phase of the new filtering compounds due to ethical problems connected to the exposure of healthy subjects to the potentially harmful effects of UV radiation. As far as the UVA Protection Factor (UVAPF) determination is concerned, in 2012, a new *in vitro* method was established and integrated into the ISO 24443:2012. Also, for *in vivo* tests, no authority or legislator has released an official statement to support all this. In the sunscreen research field, more effective guidelines for the evaluation of naturally derived actives are required. Despite the numerous scientific reports, there is no officially approved natural commercial sunfilter. Moreover, a consistent number of commercially available solar products (sunscreen) contain herbal derivatives, but an official and a widely approved validation of this method is not available, and so it is indispensable to correct labeling of the final product’s UV protection. Hence, the objective of this study is to collect any current data and exhaustive critical overview regarding the use of herbal extract and natural molecules in sunscreens. Finally, the intent of the present chapter is to provide solid types of research methodology approach in order to develop herbal or natural-based sunscreen, useful as a set of guidelines.

### 1.1. Ultraviolet radiation

Sunlight is composed of about 40% visible light (VIS), 50% infrared light (IR), and 10% ultraviolet light (UV). As far as the biological effects of solar radiation exposure are concerned, ultraviolet radiation is the most important part of the electronic spectrum. Ultraviolet (UV) radiation can be divided into UVC (200–280 nm), UVB (280–315 nm), and UVA (315–400 nm) [2].

UV radiation (sun) is essential to human health; it is necessary for the production of vitamin D3 in the skin. Vitamin D3 is necessary for the intestinal absorption of calcium and phosphorus, and deficiency may cause osteoporosis in adults and growth retardation and skeletal deformities in children. Solar radiation has other therapeutic effects on some skin diseases such as psoriasis and eczema, thus making the outdoors a healthy lifestyle choice [2, 4].

On the other hand, the negative effects of an excessive exposure to UV radiation are well known, and these are harmful to human health; the interaction of the radiation with the most

important constituents of human skin (DNA, RNA, proteins, lipids) represents the basis of the UV-mediated negative biological activities [2]. More in particular UVB radiation is responsible for the most known acute negative effect that comes after some hours of UV exposition: the UVB-induced erythema. This radiation also has a potential carcinogenic effect because it can cause direct damage to the DNA and RNA. An over exposition to deeply penetrating radiation, namely, UVA, is responsible for premature skin aging, excessive degradation, and inhibition of the synthesis of collagen fibers [5].

Protection from solar radiation thus represents a complex issue involving various biological activities and factors that influence the efficacy of a sunscreen product, as demonstrated by studies during the last decade. The most important biological activities in the field of sun protection can be summarized in six main categories:

- Filtering activity against UVB/UVA radiation
- Antioxidant and reactive oxygen species scavenging activity
- Antimutagenic activity
- Anticancer properties
- Booster effect
- Safety stability of the active compounds

In our opinion, the research effort should be focused on the discovery of multifunctional compounds or mixtures that present the abovementioned biological activities. Therefore, the reports of *in vitro* SPF values of herbal extracts and compounds are more valuable if they are accompanied by other types of useful biological activities that are of equal importance in the prevention of skin problems relating to UV exposure [2].

## **2. Biological activities connected with solar protection**

### **2.1. Antioxidant and reactive oxygen species scavenging activity**

Antioxidant effect represents one of the key mechanisms of photoprotective activity of herbal extracts. The UV skin damage depends also on the generation of reactive oxygen species (ROS). ROS are considered as oxidant agents and are responsible for the development of skin disorders like skin aging, lipid peroxidation, and cancer [6]. These species include hydroxyl radicals, peroxy radicals, superoxide anion, and, mainly, their active precursors: ozone, hydrogen peroxide, and singlet oxygen. ROS react negatively with DNA, proteins, and unsaturated fatty acids that in turn induce carcinogenic processes and inflammatory response from cells. Phenolic/polyphenolic compounds and flavonoids usually represent the main source of natural antioxidant compounds, and several types of research highlight the usefulness of including natural antioxidant extracts in topical products [6–8].

Antioxidant compounds from herbs offer new possibilities and strategies for an effective prevention and treatment of UV-mediated damages and diseases, which are mainly due to the

generation of reactive oxygen species suppressing immune reactions. The benefits of natural antioxidants in topical products are nowadays generally accepted in light of the information available [9]. There are several effective *in vitro* tests for antioxidant activity. Each of them is based on different mechanisms and, thus, evaluates different kinds of oxidative protection. In order to obtain a sufficient evaluation on *in vitro* antioxidant, it is thus necessary to perform different types of tests to assess the studied compounds on different kinds of oxidative species.

Listed below are some examples of tests that should be carried out to define the whole spectrum of protection. The tests include:

- 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay
- Luminol photochemiluminescence (PCL) assay
- 2,2'-Azino-bis-3-ethyl-benzothiazoline-6-sulfonic (ABTS) acid assay
- Oxygen radical absorption capacity (ORAC)

## **2.2. Antimutagenic activity and anticancer properties**

UV radiation is capable of damaging DNA and therefore participating in cancer pathogenesis through multiple mechanisms such as immunosuppression, oxidative stress, direct DNA damage, inflammatory response, and p53 tumor suppressor gene mutations. On the other hand, it should be taken into account that immunosuppression might be the desired effect in subjects affected by autoimmune diseases [10].

Several methods are available for a predictive *in vitro* antimutagenic or anticancer activity evaluation, and it is quite difficult to identify a list of preferred tests [11]. However, a good practice should include at least one of the validated methods in the screening of a new substance or mixture [11].

## **2.3. Anti-inflammatory activity**

UV radiation induces the inflammatory response. UVB-induced cyclooxygenase-2 (COX-2) expression leads to an increase in the production of prostaglandin (PG) metabolites. COX-2 expression in the skin has been linked to the pathophysiology of inflammation and cancer. Exposure to UV radiation is also known to increase the expression of pro-inflammatory cytokines like tumor necrosis factor, interleukin (IL)-1, and interleukin IL-6. These anti-inflammatory properties including various herbal substances and medicines can be evaluated by a number of methods [12, 13].

## **2.4. Booster effect**

This topic is yet quite complex because it is relatively new and not fully explored. There are already known compounds that can boost the SPF of UV filters [10, 14], but the mechanisms that are responsible for booster effects are heterogeneous and often unpredictable; some are linked to the nature of the UV filter(s) that the formulator wants to enhance. It is, in fact, difficult to uniquely define the general characteristics of an ingredient with booster effect, but it is possible to describe the two main aspects of this topic. The three main strategies available

to achieve “booster effect” are interaction with the UV filters at the physical-chemical level to improve efficiency (optimize the efficiency of the UV absorber mixture) [10], implement a correct formulation strategy, and improve the film-forming properties (use of emollients and film-forming agents). One of the reasons of the growing importance of the booster effect is the consolidated marketing trend of placing on the market sunscreen products with higher SPF values; as a consequence of this, the formulator has to find all the possible stratagems to use the smallest possible amount of UV filters in the product. Considering the evaluation of herbal materials as “booster ingredients,” this activity is, in some cases, identifiable by the in vitro tests that will be described later. Ingredients that improve UV filter distribution and enhance spreadability are also valuable [15].

According to EU regulation [16], Annex VI reports the list of UV filters allowed in cosmetic products.

List of sunscreen ingredients approved in the USA as presented in the sunscreen drug products for over-the-counter human use monograph” (21 CFR 352.10) [17].

### 3. Guidelines for the determination of SPF in vitro

Currently, several in vitro tests for determination of SPF exist. They are all used for screenings performed in the research and developing phase. The first method proposed is the one by Diffey (1989); it is still the most accredited reference [14]. The fundamental characteristic of all the in vitro methods is that they are based on spectrophotometric measurement of the absorbance (calculated from transmittance) of a thin film of product applied on UV transparent substrates. Substrates should be as close as possible to the physical characteristics of the skin. The amount of product applied varies from 0.7 to 2.0 mg cm<sup>2</sup>. There are different types of suitable substrates; they can range from plastic perforated surgical tape such as Transpore™ to standardized plastic plates such as polymethyl methacrylate (PMMA) plates [14]:

- Transpore™ tape: it is a surgical tape (provided by 3M Health Care Company, Maine, USA). It is used according to the Diffey-Robson’s method; this tape has a perforated structure, and it allows the distribution of the sunscreen sample in a way similar to the irregular surface of the skin.
- Sand-blasted PMMA plates: this substrate is easy to use and can be supplied with a reproducible roughness. (i.e., Schonberg GmbH, Munich, Germany). The plates have an area of 2 cm<sup>2</sup> and standard roughness of 5 μm. The features of this substrate meet the recommendation of ISO 24443 for in vitro UVA protection assessment.

Our experience in the research of useful compounds in the solar protection field and an accurate bibliographic research indicate that it is possible to point out several factors and variables which are able to affect the accuracy and the repeatability of the in vitro SPF tests. The most important ones are:

- Different compositions of filters
- The formulation of the sunscreens

- The thickness and the homogeneity of the applied sunscreen
- The type of spectrophotometer
- Substrates used and their relative roughness

The main concern, about this type of evaluation, is the lack of data to support correlation to *in vivo* results [14].

At present, the *in vivo* method is still the official standard for UVB protection (ISO 2444:2010), and product developers should perform the *in vivo* test on the final product and the *in vitro* one during all the phases of the development bringing attention to the ethical issue and on the costs.

In order to provide practical indications, we suggest two methods that have proven, in our experience, to be among the most reliable:

### 3.1. Method A

This is based on the Diffey-Robson's method [14]. The support used is a Transpore™ surgical perforated tape, cut to have an area of 20 cm<sup>2</sup>, in which an amount of  $0.0400 \pm 0.002$  g (2 mg cm<sup>-2</sup>) of the product is weighed and laid in small spots through all the area. The tape is then positioned on a scale where the spreading phase is carried out with a finger cot, performing a pattern of six movements in horizontal, vertical, and circular directions and checking the pressure applied in all the movements. As far as the spreading pressure is concern, an internal procedure must be developed by the performing laboratory in order to be repeatable (see the end of this paragraph). At least three tapes have to be prepared for each product, recording five measures each, collecting therefore 15 spectra [14].

### 3.2. Method B

This method has been recently proposed by us [14], adapting to UVB the ISO 24443:2012 standard for the *in vitro* UVA protection determination. The support used is a PMMA (polymethyl methacrylate) plastic plate with an area of 25 cm<sup>2</sup> and standardized 5 μm roughness, in which an amount of  $0.0320 \pm 0.0005$  g (1.3 mg cm<sup>-2</sup>) of the product is weighed and laid in small spots through all the area. The plate is then positioned on a scale where the spreading phase is carried out performing with pre-saturated finger cot a sequence of six movements in horizontal, vertical, and circular direction and checking the pressure applied throughout the spreading. Before the measurement, the sample lies for a minimum of 15 min in a dark place, allowing the evaporation of volatile components. Three plates have to be prepared for each product, recording five measures each, collecting therefore 15 spectra [14].

In both methods, the spectra were recorded with an appropriate spectrophotometer, wavelength ranging from 290 nm up to 400 nm, with increment step set at 1 nm. The tests carried out for the evaluation of new herbal compounds are usually performed including them, at a known concentration, in a stable formulation suitable for cosmetic use. The obtained SPF data are then compared to those of the same formulation without the studied compounds.

SPF in vitro is defined as follows:

$$In\ vitro\ SPF = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda)I(\lambda)d(\lambda)}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda)I(\lambda)10^{-A(\lambda)}d(\lambda)} \quad (1)$$

$E(\lambda)$  is the erythema action spectrum (CIE-1987) at the wavelength  $\lambda$ .  $I(\lambda)$  is the spectral irradiance received from the UV source at the wavelength  $\lambda$ .  $A(\lambda)$  is the monochromatic absorbance of the test product layer at the wavelength  $\lambda$ .  $d(\lambda)$  is the wavelength step (1 nm).

Both methods have to be conducted in highly standardized operating conditions with regard to the operator, the environmental conditions, the substrates used, and the instruments. We have worked with spreading pressures of  $100 \pm 15$  g and  $200 \pm 15$  g, and comparing different application pressures on the same substrate, no statistically significant difference subsists in terms of repeatability.

The two fundamental parameters in the in vitro SPF measurement process are in vivo correlation and reproducibility. In our experience, Method B with a spreading pressure of  $200 \pm 15$  g is the most reliable method with respect to reproducibility and accuracy. Nevertheless, Method A can be still considered as a useful in vitro method during the early research phase, especially in laboratories with limited financial resources and limited equipment. In this case, the correlation is not influenced by the choice of operator's pressure ( $100 \pm 15$  g or  $200 \pm 15$  g).

The problem of photostability of UV filters should also be considered at this stage. It is necessary, seeking potential human applications, to verify that a new compound or vegetal extract does not present any photostability problems. This evaluation can be performed using solar radiation simulators; this procedure is also indicated in the ISO 24443:2012.

It is very important to assume that, at present, it is possible for a single laboratory to optimize internal methods and protocols to achieve repeatable and predictive in vitro results, whereas it is extremely difficult to develop methods reproducible and equally reliable between different laboratories due to external variables (e.g., the environmental, operator, etc.) [14].

#### 4. Natural compounds in solar radiation protection: current knowledge

In recent years, many plant species have been investigated for their potential uses in the field of solar radiation protection, but much remains to be accomplished. As stated above, this depends on both a large number of under-investigated species and the lack of an official standard in vitro SPF evaluation method, to speed up the screening procedure. Depending on this, and on the many different and incomplete approaches led by different research groups, it is also complex to have a general picture of the existing knowledge. In the aim to achieve a "state of the art," we conducted a detailed bibliographic research on the plants already investigated in biological activities useful for sunscreen products.

In our previous investigation [2], we identified 54 plants, 5 lichens, and 14 pure molecules which have been studied in order to obtain herbal sunscreen products. It is remarkable how



many plant extracts showed preliminary natural UV filter activity and, in the same manner, antioxidant properties and/or synergistic photoprotective effects.

**Table 1** summarizes a selection of the abovementioned plants, lichens, and pure molecules which have been mentioned at least in two different types of research.

| Plant name                        | Plant part(s) used     | Plant extract           | Type of compound(s)                                      | Major constituent(s)  | Main effect(s)   |
|-----------------------------------|------------------------|-------------------------|--|---|--|
| <i>Calendula officinalis</i>      | Flower                 | Hydroalcoholic extract  | Polyphenol, flavonoid                                    | Rutin, narcissin  | Prevent UV irradiation-induced oxidative stress  |
| <i>Camellia sinensis</i>          | n.r.                   | n.r.                    | Polyphenols  | EC-(−)epicatechin, ECG-(−)-epicatechin-3-gallate, EGC-(−)-epigallocatechin, EGCG-(−)-epigallocatechin-3-gallate | Anticarcinogenic, anti-inflammatory, photostabilizing capacity   |
| <i>Coffea</i> genus (10 species)  | Green dry coffee beans | Chloroform extract      | Lipid fraction   | Linoleic acid, palmitic acid  | UV absorber, emollient   |
| <i>Culcitium reflexum</i>         | Leaf                   | Ethanol extract         | Phenolic compounds, flavonols                            | Rutin, kaempferol, quercetin, and its glycosylated derivatives and cinnamic acid derivatives                    | Antioxidant, reduces UVB-induced skin erythema, free-radical-scavenging effect   |
| <i>Fragaria x ananassa</i>        | Fruits                 | n.r.                    | Anthocyanins and hydrolyzable tannins                    | Pelargonidin  | Antioxidant, reduces UVB-induced skin erythema, anti-inflammatory, diminishing DNA damage on UVA-induced skin damage                 |
| <i>Glycine max</i>                | Seeds                  | Soybean cake            | Soy isoflavone   | Genistein   | Antioxidant, reduces skin photo damage and transepidermal water loss (TEWL)  |
| <i>Moringa oleifera</i>           | Seeds                  | Petroleum ether extract | Lipid fraction   | n.r.  | UV absorber  |
| <i>Pinus pinaster</i>             | Bark                   | Picnogenol              | Phenolic compounds, polyphenols, procyanidin derivatives | Catechin, epicatechin, taxifolin, caffeic, ferulic, p-hydroxybenzoic, vanillic, gallic, and protocatechuic acid | Reduces UVB-induced skin erythema, free-radical-scavenging effect  |
| <i>Pimenta pseudocaryophyllus</i> | Leaves                 | Ethanol extract         | Flavonoids and polyphenolic compounds                    | n. r.   | Inhibits UV-B irradiation-induced inflammation and oxidative stress of the skin Antioxidant, decreases oxidative damages of the skin |

| Plant name                    | Plant part(s) used | Plant extract               | Type of compound(s)                  | Major constituent(s)   | Main effect(s)  |
|-------------------------------|--------------------|-----------------------------|--------------------------------------|--|---|
| <i>Pongamia glabra</i>        | Seeds              | n.r.                        | n.r.                                 | Pongamol, karanjin   | UV absorber   |
| <i>Punica granatum</i>        | Fruits, peel       | methanol extract            | Anthocyanidins, hydrolyzable tannins | Delphinidin, cyanidin, and pelargonidin                                      | Decreases in the number of UVB-induced dimers in the human skin, synergic photoprotective activity in nanostructured lipid carrier        |
| <i>Silybum marianum</i>       | Seeds              | n.r.                        | Flavonolignans                       | Silymarin, Silybin, silydianin, silychristin, isosilybin                     | Inhibits UVB-induced damage, antioxidant  |
| <i>Vaccinium myrtillus</i> L. | Fruits             | n.r., water-soluble extract | Polyphenols, anthocyanins            | n.r.   | Reduction of UV A-stimulated ROS formation, attenuation of UVA-caused peroxidation of membrane lipids, and depletion of intracellular GSH |
| <i>Vitis vinifera</i>         | Seeds              | n.r.                        | Polyphenols                          | Flavan-3-ol derivatives, catechin, epicatechin, oligomeric proanthocyanidins | Free-radical-scavenging effect, prevents UVB- and UVC-induced lipid peroxidation, reducing the oxidative stress and apoptosis             |

n.r.—not reported.

**Table 1.** Selection of plant extracts useful for sunscreen application.

Lichens are mentioned by several types of research [18–23] as natural sources of photoprotective compounds and phytocomplexes. Some examples of bioactive compounds obtained by lichens are epiphorelic acid I and II, salazinic acid, usnic acid, secalonic acid, and calycine. According to the studies regarding lichens, the main highlighted activities were antioxidant properties and broad-spectrum UV-absorbing capacity. Regarding pure molecules, quercetin and resveratrol have been widely investigated [7, 24–30] for their antioxidant, antiproliferative, and anti-inflammatory properties, including also UVA and UVB filter enhancer activity.

## 5. Strategies and solutions

UV light is recognized by the US National Institute of Environmental Health Sciences, as the main etiological agent of a large number of skin cancers, sunburns, and oxidative stress (US Tenth Report on Carcinogens). Despite controversial data about photo-irritation, photosensitization, and contact dermatitis, synthetic and mineral sunscreens are used to prevent UV-induced skin damage and are very common in several skin care formulations. More often than not, the etiology of a skin disease is multifactorial and includes DNA damages, inflammatory

processes, oxidative stress from ROS, lipid peroxidation, etc. All the abovementioned causes need a multi-target approach, which is impossible to obtain with a “magic-bullet” molecule and neither in a blend of UVA/UVB/UVC filters. All synergic photoprotective claims may be integrated with a proven formulation strategy (oleosomes and/or other encapsulation technologies, coatings or stabilizers, film thickness, etc.) in order to stabilize and/or boost the sun care herbal ingredients.

Moreover, in view of the increased demand of natural, herbal ingredients, sunscreens will be the next trend for photoprotective formulations. To this end, it is mandatory to develop natural sunscreen formulations based on a sound scientific investigation to sustain safe and effective products. In these regards, in our laboratory, we have very recently developed a rational approach considering the synergistic properties that a good candidate should possess: proven UVB/UVA absorption capability, antioxidant effects, protection against DNA and other free radical cellular structure-mediated damages, potential synergic protective mechanisms, and, finally, good toxicological profiles and proven formulation efficacy. As a matter of fact, several herbal/natural molecules may provide, in theory, these activities. Herbal extracts are naturally composed of mixtures of synergistic ingredients developed by plants through the evolution process (i.e., polyphenols), to allow the earlier marine organisms to colonize the terrestrial environment inferring resistance to high UV-induced oxidative stress. Taking this into account, as the synthetic strategy goes toward the design of multifunctional molecules inspired by the above natural mechanism [7], herbal sunscreen goes in the same direction but starts from the other side that already is available in the phytocomplex. The weak point of this latter approach is the investigation strategy, often incomplete, that makes literature data not useful. To further complicate the picture, it must be noted that different portions of the plant may be used (leaves, bark, roots, flowers, seeds, or fruits), they can be dried either in air or using instruments, and they can be cut or ground into particles and then extracted with either water or organic solvents at different herbs to solvent ratio (i.e., 1:4, w/v). But also fresh herbs are used; in this case, the herb to solvent ratio is usually 1:1. The extraction process might be different in function of the characteristics (physical and chemical) of the ingredients. Thus, the same approach for different compounds cannot be devised. Also, steam distillation is used in the preparation and extraction of essential oils from botanical materials. New technologies, i.e., supercritical carbon dioxide extraction technology, membrane separation technology, enzymatic extraction methods, and so on, are emerging, further complicating the pattern of the relative observed activities.

Taking all this into account, only a few studies on herbal extracts really match the abovementioned strategy. An ideal approach (**Figure 1**) should involve three different steps, typical of drug development:

1. Extraction and characterization of the properties of the extracts (i.e., composition, UV absorption, mutagenicity, cytotoxicity).
2. In vitro evaluation of synergic physiological activity (i.e., lenitive, antiradical, antioxidant, etc.).
3. Formulation strategies, new vehicles development, stabilization, and SPF evaluation in vitro and on volunteers.

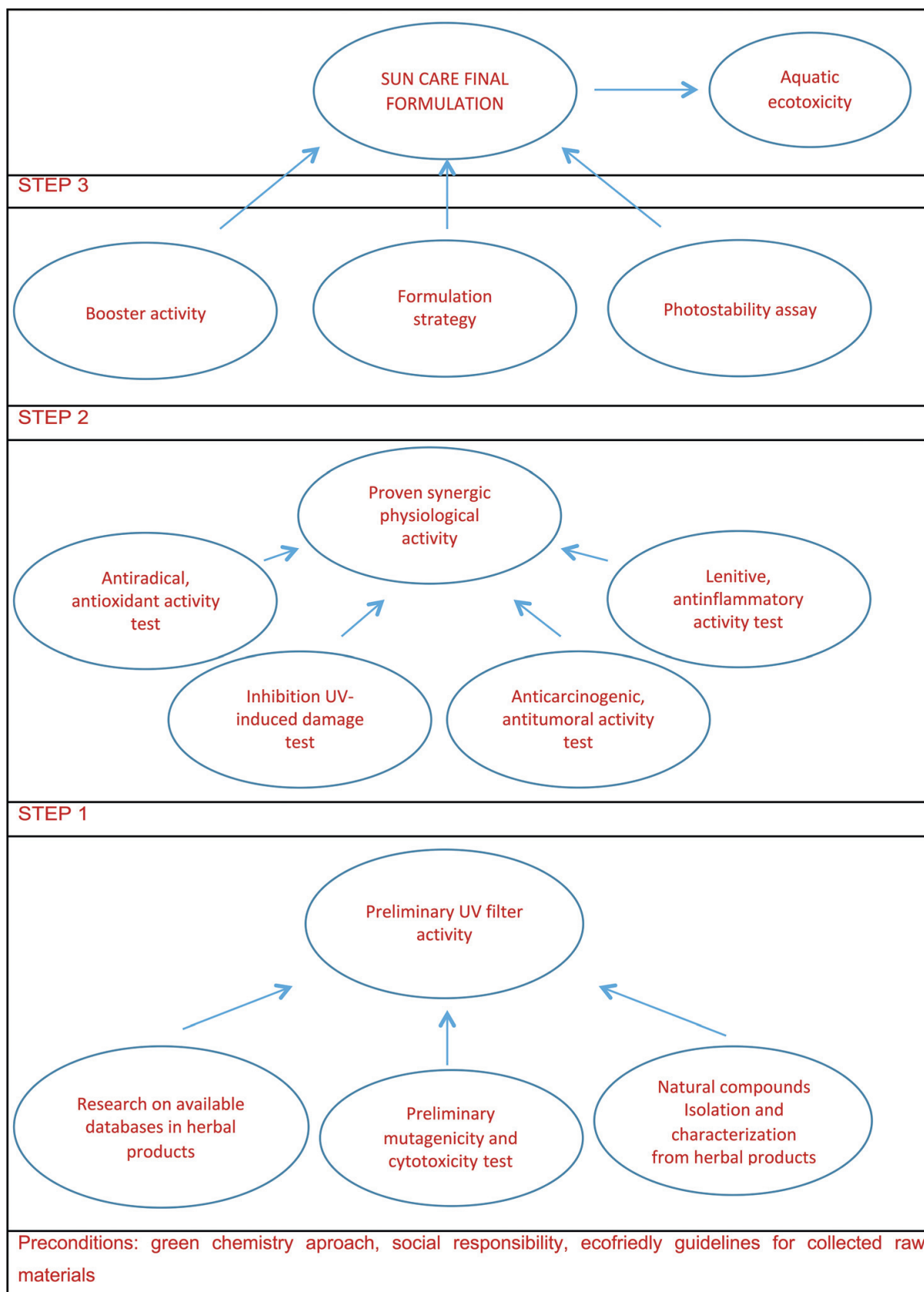


Figure 1. A rational process in sunscreen active ingredients discovery from herbal extracts.

In our opinion, step 3 represents the main lack of evidence in order to develop a natural sunscreen product. Despite an improving number of recent studies regarding the incorporation of antioxidants into sunscreen [25, 27, 28], none of the researches we reviewed include herbal products and more sophisticated formulations, as nanostructured lipid carrier, elastic niosomes, nanoparticles, microemulsion, etc. We recommend this way as an essential trend for “green” sunscreen research.

## 6. Natural extracts as a source of active compounds: from ancient to modern times

Scientific reports directed to the discovery of novel natural photoprotective ingredients often describe only the UV-filtering activity (step 1), which is a necessary but not sufficient condition to support the speculation of the effectiveness if inserted into a sunscreen tested on volunteers. The same issue can be referred to the *in vitro* bioactivity studies (step 2). The hardest challenge is to enhance the already approved findings as models, mentioned in step 3. An extract or natural compound needs to be fully characterized also for its effect in humans in the final product. Without this, it will remain restricted to a scientific investigation, which will be seen as useless to understand the potential of application in substitution of synthetic or mineral filters. Finally, in order to demonstrate real “green” claims, we recommend completing the product development with aquatic ecotoxicity assay. This step is becoming quite relevant [31, 32] and could be a significant benefit for a new sunscreen product.

Natural extracts have often been used as a source of inspiration in the development of new drugs rather than drugs themselves. Thus, while the discovery of synthetic ingredients is based on a rational systematic approach, which takes into account “step-by-step” modifications driven by chemical-physical parameter, the approach to the discovery of herbal ingredients, to be used as extracts, is “experience driven” and mainly based on traditional uses. A step-by-step procedure applied to natural extract would imply (I) the preparation of extracts and eventually phytochemicals from herbs, (II) the phytochemical study of extracts of herbal preparation or compound isolation, (III) the structure/composition elucidation, (IV) the *in vitro* biological activity evaluation, (V) the compound characterization and principal activity investigation, (VI) and the *in vivo* proof of the *in vitro* elucidated activities.

Furthermore, on the one hand, a central government agency of countries with high biodiversity should consider establishing research projects that involve ecological ethics, such as the managing, care, and preservation of the environment. However, on the other hand, the discovery of such ingredients could lead to improving agriculture or farming of these plants which may become an important job opportunity, especially in countries where the land is not favorable for the traditional farming. Finally, biotechnology in fields of plants is already a precious source of ingredients (i.e., secondary metabolites), which can be obtained from cell culture rather than traditional farming, thus saving biodiversity and land to be dedicated to plants for food. This has already been proven possible in the field of medicinal plants (i.e., *Artemisia annua*) [33].

Due to the growing interest in herbal remedies, there is also a significant amount of data available on herbal ingredients (i.e., public databases containing analysis, efficacy tests, extracts preparation) even in relation to their molecular targets [33].

It is already possible, based on existing proofs, to envisage a stage of discovery from herbal ingredients, which includes the preparation of extract (by the same standardized methods) eventual isolation, structure/composition elucidation, and *in vitro* bioactivity evaluation. In the case of sunscreens, the class of compounds behaving abilities of solar radiation absorption and antioxidant capacities are well known (i.e., polyphenols); what is not known is how much the mixture of other ingredients present in the extract may contribute to the sunscreen activity with complementary mechanisms (i.e., booster activity). This implies that the evaluation of activity *in vivo* must be conducted for each single extract. As recently reviewed by Si-Yuan Pan et al., the herbal preparation may contain “hundreds” of active compounds, and in addition, the concentrations of some of them might be exceedingly low and thus insufficient for conducting *in vivo* studies on isolated molecules. They report that from 1960 to 1982 and from over 100,000 crude tested extracts (deriving from more than 30.00 plants) only two compounds, Taxol and camptothecin, were developed into marketable therapeutics [33].

Based on the experience from random trials and observations in animals, ancient people acquired the knowledge of using herbs to treat illnesses. However, herbs used in traditional medicines constitute only a small portion of naturally occurring plants; thus, a large part of work still remains to be developed.

## 7. Conclusion

In this chapter, we presented a systematic approach based on our experiences and proposed it as a possible standard approach in this field. The steps mentioned in **Figure 1** have to be considered as an initial set of guidelines needed for the development of herbal-based sunscreen. The end result is a complete and rational methodology for the research and development of herbal sunscreen. The authors consider it to be essential to match the initial *in vitro* studies about UV filter activities with synergic biological activities (antioxidant, anti-inflammatory, inhibitory UV-induced damage effect, etc.) and formulation strategies (boosters, encapsulation, etc.). A solid response in each step may be considered a complete strategy. Finally, regarding natural products and traditional knowledge, an eco-friendly and sustainable approach can complete the investigation process and the management of the industrial supply chains.

We believe that our contribution will be useful to expedite the discovery of sunfilters from herbs. With a solid discovery approach, chances of success will greatly increase.

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## A. Annex VI: List of UV filters allowed in cosmetic products according to EU regulation [11]

List of sunscreen ingredients approved in the USA as presented in the “Sunscreen Drug Products for Over-the-Counter Human Use” monograph (21 CFR 352.10) [12].

| Chemical name   | Name of common ingredient glossary        | Maximum concentration in ready-for-use preparation |
|---|---|--|
| 4-Aminobenzoic acid   | PABA                                      | 5%   |
| <i>N,N,N</i> -Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulfate  | Camphor benzalkonium methosulfate         | 6%   |
| Benzoic acid, 2-hydroxy-, 3,3,5-trimethylcyclohexyl ester/homosalate  | Homosalate                                | 10%  |
| 2-Hydroxy-4-methoxybenzophenone/oxybenzone  | Benzophenone-3                            | 10%  |
| 2-Phenylbenzimidazole-5-sulfonic acid and its potassium, sodium, and triethanolamine salts/ensulizole   | Phenylbenzimidazole sulfonic acid         | 8% (as acid)                                       |
| 3,3'-(1,4-Phenylenedimethylene) bis(7, 7-dimethyl-2-oxobicyclo-[2.2.1]hept-1-yl-methanesulfonic acid) and its salts/ecamsule                              | Terephthalylidene dicamphor sulfonic acid | 10% (as acid)                                      |
| 1-(4-tert-Butylphenyl)-3-(4-methoxyphenyl)propane-1,3-dione/avobenzone  | Butyl methoxydibenzoylmethane             | 5%   |
| alpha-(2-Oxoborn-3-ylidene)-toluene-4-sulphonic acid and its salts  | Benzylidene camphor sulfonic acid         | 6% (as acid)                                       |
| 2-Cyano-3,3-diphenyl acrylic acid, 2-ethylhexyl ester/octocrylene   | Octocrylene                               | 10% (as acid)                                      |
| Polymer of N-((2 and 4)-((2-oxoborn-3-ylidene)methyl)benzyl)acrylamide  | Polyacrylamidomethyl benzylidene camphor  | 6%   |
| 2-Ethylhexyl 4-methoxycinnamate/octinoxate  | Ethylhexyl methoxycinnamate               | 10%  |
| Ethoxylated ethyl-4-aminobenzoate   | PEG-25 PABA                               | 10%  |
| Isopentyl-4-methoxycinnamate/amiloxate  | Isoamyl p-methoxycinnamate                | 10%  |
| 2,4,6-Trianiilino-(p-carbo-2'-ethylhexyl-1'-oxy)-1,3,5-triazine   | Ethylhexyl triazine                       | 5%   |
| Phenol,2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl)                                    | Drometrizole trisiloxane                  | 15%  |
| Benzoic acid, 4,4-((6-(((1,1-dimethylethyl)amino)carbonyl)phenyl)amino)-1,3,5-triazine-2,4-diyl)diimino)bis-, bis(2-ethylhexyl) ester/iscotrizinol (USAN) | Diethylhexyl butamido triazine            | 10%  |
| 3-(4-Methylbenzylidene)-d1 camphor/enzacamene   | 4-Methylbenzylidene` camphor              | 4%   |
| 3-Benzylidene camphor   | 3-Benzylidene camphor                     | 2%   |

| Chemical name   | Name of common ingredient glossary                  | Maximum concentration in ready-for-use preparation |
|---|---|--|
| 2-Ethylhexyl salicylate/octisalate  | Ethylhexyl salicylate                               | 5%   |
| 2-Ethylhexyl 4-(dimethylamino)benzoate/padimate O (USAN: BAN)   | Ethylhexyl dimethyl PABA                            | 8%   |
| 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid and its sodium salt/sulisobenzone                         | Benzophenone-4, benzophenone-5                      | 5% (as acid)                                       |
| 2,2'-Methylene-bis(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol)/bisotrizole               | Methylene bis-benzotriazolyl tetramethylbutylphenol | 10%  |
| Sodium salt of 2,2'-bis(1,4-phenylene)-1H-benzimidazole-4,6-disulfonic acid/bisdisulizole disodium (USAN) | Disodium phenyl dibenzimidazole tetrasulfonate      | 10% (as acid)                                      |
| 2,2'-(6-(4-Methoxyphenyl)-1,3,5-triazine-2,4-diyl)bis(5-((2-ethylhexyl)oxy)phenol)/bemotrizinol           | Bis-ethylhexyloxyphenol methoxyphenyl triazine      | 10%  |
| Dimethicodiethylbenzalmalonate  | Polysilicone-15                                     | 10%  |
| Titanium dioxide  | Titanium dioxide                                    | 25%  |
| Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester  | Diethylamino hydroxybenzoyl hexyl benzoate          | 10% in sunscreen products                          |

| Name of common ingredient glossary | Concentration allowed |
|------------------------------------|-----------------------|
| Aminobenzoic acid (PABA)           | Up to 15%             |
| Avobenzone                         | Up to 3%              |
| Cinoxate                           | Up to 3%              |
| Dioxybenzone                       | Up to 3%              |
| Homosalate                         | Up to 15%             |
| Menthyl anthranilate               | Up to 5%              |
| Octocrylene                        | Up to 10%             |
| Octyl methoxycinnamate             | Up to 7.5%            |
| Octyl salicylate                   | Up to 5%              |
| Oxybenzone                         | Up to 6%              |
| Padimate O                         | Up to 8%              |
| Phenylbenzimidazole sulfonic acid  | Up to 4%              |
| Sulisobenzone                      | Up to 10%             |
| Titanium dioxide                   | Up to 25%             |
| Trolamine salicylate               | Up to 12%             |
| Zinc oxide                         | Up to 25%             |
| Ensulizole                         | Up to 4%              |
| Homosalate                         | Up to 15%             |



| Name of common ingredient glossary | Concentration allowed |
|------------------------------------|-----------------------|
| Meradimate                         | Up to 5%              |
| Octinoxate                         | Up to 7.5%            |
| Octisalate                         | Up to 5%              |
| Octocrylene                        | Up to 10%             |
| Oxybenzone                         | Up to 6%              |
| Padimate O                         | Up to 8%              |

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