Effects of Natural Products on Contact Dermatitis

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Abstract: Some medicinal plants, which are known to produce allergic reactions, are also specifically used as anti-inflammatory agents. Among the more relevant plants, we report species with cinnamaldehyde, cinnamic alcohol, geraniol, hydroxycitronellal, eugenol and isoeugenol are all potential allergens. In addition, fragrances, which are mixtures of small-molecular-weight compounds, may induce allergic contact dermatitis due to fragrance-specific $CD4^+$ and $CD8^+$ T lymphocytes. Plants from the Asteraceae family used in folk medicine as anti-inflammatories can cause allergic contact dermatitis because of its content in sesquiterpene lactones, which have been reported as the anti-inflammatory principles in this species.

Species with flavonoids, iridoids, terpenoids and alkaloids have been described as inhibitors of contact dermatitis. *Scrophularia auriculata*, *Poria cocos*, *Santolina chamaecyparissus*, *Ranunculus sceleratus* and *Helichrysum italicum* all showed activity in different experimental protocols of contact dermatitis, thus justifying the potential use of these medicinal plants as anti-allergens and inhibitors of contact dermatitis reactions produced by allergens and chemicals.

Hydroquinone derivatives such as 1-*O*-β-glucopyranosyl-2-(3'-hydroxymethyl-3'-methylallyl) hydroquinone and arbutin, flavonoids such as kaempferol, apigenin and genistein, sesquiterpene lactones such as helenalin, diterpenes such as triptonide, triterpenes such as tripterine and bryonolic acid, iridoids such as scrovalentinoside, alkaloids such as indirubin, dehydrocorydaline, magnoflorine hydroxide and phellodendrine acetate, and polysaccharides such as fucoidin have been reported as inhibitors of contact dermatitis reactions.

Key words: contact dermatitis; anti-allergenic; medicinal plants; natural products; phenolics; flavonoids; terpenoids; alkaloids.

INTRODUCTION

Dermatitis is a polymorphic inflammation of the skin. The acute phase is characterized by redness, itching, swelling and blistering, with formation of crusts and scales during the healing process. The chronic phase is characterized by dryness, hyperkeratosis and fissures. There are multiple forms of dermatitis, the most common being atopic dermatitis and contact hypersensitivity [1]. Although they are completely different in nature and pathogenetically unrelated, in their acute phases both pathologies exhibit common morphological features, notably vesicle formation, which cause the breaching of the epidermal barrier and the consequent loss of the protective function of the epidermis [2].

One possible manifestation of contact hypersensitivity is contact dermatitis, an altered state of skin reactivity induced by exposure to an external agent. The clinical presentation may vary depending on the identity of the triggering agent and the reactivity of the subject, but in all cases the lesions are primarily located in the place of contact. Four types of contact reactions may be distinguished according to the mechanism of elicitation: allergic contact dermatitis, which is a type-IV T-cell-mediated reaction [3]; irritant contact dermatitis; phototoxic and photoallergic contact dermatitis; and immediate type contact reactions [4,5].

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Contact dermatitis can be acute, subacute, or chronic, and each of these phases may progress into the next [6]. Eczema and dermatitis are often used synonymously to describe a polymorphic pattern of inflammation of the skin characterized, at least in its acute phase, by erythema, vesiculation and pruritus [5]. Allergic contact dermatitis is brought on by exposure to substances such as chemicals, cosmetics, fragrances, and preservatives. Avoidance of the inducing agent is usually curative, although in some situations this is difficult. Symptomatic treatment usually involves preferably topical administration of emollients, moisturisers, antihistamines, and corticosteroids [6].

Although the induction mechanisms for irritant and allergic contact dermatitis are very different, the pathologies are extremely similar both clinically and histologically. Biopsies taken from allergic and irritant patch-test reactions showed similar increases in interleukin (IL)-1α, IL-2, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, as compared to normal skin. In addition, intercellular adhesion molecule-1 (ICAM-1) expression is stimulated by TNF-α in both allergic and irritant contact reactions [7].

ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis is a form of delayed-type hypersensitivity (DTH) in which the allergen first provokes a sensitization phase and then, after exposure to the same compound, a latter elicitation phase. The response is mediated by a series of cellular and molecular mechanisms. After topical exposure to susceptible amounts of a given allergen an immune response is induced. Epidermal

1568-0142/05 $50.00+.00

Langerhans cells and other cutaneous dendritic cells transport the allergen from the skin to regional lymph nodes, where it is presented to T lymphocytes [1,8,9]. Before this can occur, however, protein antigens must be broken down into small peptides by the antigen-presenting cells (APC) such as dendritic cells, macrophages and monocytes. The peptide fragments resulting from this metabolism are presented on the surface of the APCs and bound to major histocompatibility complex (MHC) molecules [10].

In the elicitation phase, once the skin has been exposed to a new contact either in the same location or elsewhere, an immune response causes a considerable reaction at the point of contact [8]. This reaction is characterized by the infiltration of T lymphocytes into challenged skin sites and the development of a cutaneous inflammation [9].

While the elicitation response in allergic contact dermatitis is dose dependent, the reaction depends on individual sensitivity as well as the exposure concentrations. Thus, when the concentrations of the allergen or the degree of sensitivity are low, the contact dermatitis may develop only after several weeks of exposure [11].

Cells Implicated in Contact Dermatitis

Dendritic cells are a heterogeneous cell population present in peripheral tissues, principally in skin and mucosae. They take up self and non-self antigens, which are then processed into proteolytic peptides, and then loaded onto MHC class I and II molecules. Peripheral dendritic cells tend to perform this task quite inefficiently, but external signals such as inflammatory cytokines induce the maturation of dendritic cells, transforming them into efficient APCs and T-cell activators [12].

Langerhans cells, which comprise a class of follicular dendritic cells, are the principal APCs involved in contact hypersensitivity. Located in the suprabasal epidermis, they are derived from the bone marrow and are responsible for the expression of CD1 and MHC class II antigens. They also have superficial receptors for IgG (FcγR) and complement C3b (CR1) [13].

Contact hypersensitivity reactions, as a form of DTH, are mediated by lymphocytic effector cells. Until recently, the prevailing view held that allergic contact dermatitis was affected by $CD4^+$ Th1-type cells; however, it has recently been demonstrated that other effectors, including $CD8^+$ T lymphocytes, are also implicated [14]. The major qualitative difference between $CD4^+$ and $CD8^+$ T cells is that the former recognize antigens presented by class II MHC molecules, whereas the second recognize antigens presented by class I MHC molecules. In addition, $CD4^+$ T cells tend to produce higher levels of cytokines, such as IL-4 and IL-5, which are both implicated in the production of antibodies by B cells [10].

Gocinski and Tigelaar [15] illustrated the differences between contact hypersensitivity reactions by examining the DTH reaction in mice. In general, DTH reactions are mediated by $CD4^+$ cells and down regulated by $CD8^+$ cells, but in contact hypersensitivity, the opposite occurs – reactions are mediated by $CD8^+$ cells and down regulated by $CD4^+$ cells. Moreover, mice lacking MHC class I antigens were unable to support contact hypersensitivity responses to dinitrofluorobenzene (DNFB), whereas MHC class II-deficient mice exhibited an exaggerated contact hypersensitivity reaction [16]. However, studies on peptides derived from trinitrophenyl with affinity to class I or class II MHC antigens revealed that while $CD8^+$ are potent effectors of contact hypersensitivity, $CD4^+$ are also active [17]. In addition, studies with DNFB and oxazolone in genetically manipulated Knockout mice demonstrated that both $CD4^+$ Th1 and $CD8^+$ Tc1 cells are crucial effectors in contact hypersensitivity [18]. In conclusion, while it seems that Tc1-type phenotype cells are the most relevant effectors of $CD8^+$ lymphocytes, other cells may also contribute to the development of allergic contact dermatitis [9].

In essence, DTH recruits effector αβ-T cells into peripheral tissues, which are then activated by APCs to produce pro-inflammatory cytokines. It is assumed that the αβ-T cells are responsible for the actual DTH reaction, but three other lymphocyte subsets are involved, two T cell types, the assisting γδ-T cells and the inducing natural killer (NK) T cells, as well as the initiating B-1 cells [19]. Effector αβ-T cells are essential, but cannot be recruited into the tissues without the local action of IgM antibodies that are rapidly produced by B-1 cells after immunization [20]. The process may be summed up in the following steps: in the elicitation phase or contact sensitivity initiation, the IgM forms a complex with the challenge antigen to locally activate the complement, which leads to the vascular activation required for T cell recruitment. The essential contact sensitivity-inducing NK T cells then activate the B-1 cells by rapidly producing IL-4 after immunization, after which contact sensitivity cells assist the local inflammatory function of the recruited contact sensitivity-effector αβ-T cells [19]. The mechanism of B cell involvement in contact sensitivity-initiation occurs through local C5a generation, which activates local mast cells to release serotonin and TNF-α to induce endothelial ICAM-1 and vascular cell adhesion molecule (VCAM-1), which in turn leads to T cell recruitment [20].

Mediators

The resulting activation of the T cells produces cytokines such as chemokines for macrophages, other T cells and, to a lesser extent, neutrophils, as well as TNF-β and IFN-γ. The consequence is a cellular infiltrate in which mononuclear cells such as T cells and macrophages tend to predominate.

The epidermis is a rich source of cytokines, some of which are expressed constitutively, whereas others must be induced by an appropriate stimulus [21]. Some cytokines are being produced by either Langerhans cells or keratinocytes, or by both [22]. The epidermal cytokines that play the most relevant role in Langerhans cell mobilization are TNF-α, IL-1β, and IL-18, and granulocyte/macrophage colony-stimulating factor (GM-CSF) in maturation [8,21]. IL-1β is an important and relevant essential mediator for the initiation of cutaneous immune responses and its upregulation by Langerhans cells may represent a mandatory early step in skin sensitization. Moreover, the IL-1β mRNA expression by dendritic cells may be a possible basis for an alternative approach to skin sensitization hazard identification [21].

Other epidermal cytokines, notably IL-10, can also modulate Langerhans cell migration [8]. Some cytokines are produced by both Th1 and Th2 cells, while others are associated with only one of them. For example, Th1 cells produce IL-2, IFN-γ, and TNF-α, while Th2 cells preferentially secrete IL-4, IL-5, IL-9, IL-10, and IL-13 [8,23]. Both Th1 and Th2 cells can produce IL-3 and GM-CSF [23].

Strong contact with sensitizers such as DNFB, dinitrochlorobenzene (DNCB), or oxazolone has been shown to induce lymph node cell population of $CD8^+$ T lymphocytes, causing an increase in the levels of IFN-γ, which plays a central role in inflammatory cutaneous reactions, and of $CD4^+$ T cells, which produce IL-4 and IL-10, but no detectable amount of IFN-γ [24,25]. Moreover, high concentrations of IFN-γ from $CD4^+$ were observed after exposure to the contact allergen formaldehyde [25]. From these results, as well as those from complementary experiments, it can be deduced that $CD8^+$ T cells, which are responsible for IFN-γ production, mediate skin sensitization, while $CD4^+$ T cells, which produce IL-4 and IL-10, limit the magnitude and the duration of the response [26].

Different skin cells, such as keratinocytes, Langerhans cells, melanocytes, fibroblasts, and endothelial cells, are capable of synthesizing nitric oxide (NO). This process is mediated by several NO synthases (NOS), including the inducible form of NOS (iNOS) [27]. NO play a relevant role in contact dermatitis, causing inflammatory responses in the implicated tissues; however, the mechanisms by which NO acts in contact dermatitis remain unclear. Ross and Reske-Kunz [1] proposed a model of the role of NO in the elicitation phase of contact dermatitis. Thus, during the first phase, after elicitation by allergen contact, there is an unspecific phase characterized by low NO-levels in which both vasodilatation and attraction of neutrophils take place. Subsequently, the antigen specific phase develops, marked by high NO-levels which lead to inhibition of mediator release by the mast cells, down regulation of adhesion molecules, disruption of neutrophil attraction, and apoptosis of effector cells.

Different studies have demonstrated that expression of iNOS is induced in contact dermatitis and that the corresponding inhibitors of iNOS suppress the contact hypersensitivity responses. However, iNOS-deficient mice develop an aggravated contact dermatitis response late in the elicitation phase [1]. Suppressors of iNOS synthesis, e.g. corticosteroids, could serve as a therapy for contact dermatitis, but selective suppression of iNOS synthesis might obtain similar results while avoiding the negative side effects of corticosteroids [28]. Unfortunately, the extent of suppression of iNOS expression in animal models was not clear in the research conducted thus far, with only local inhibition of iNOS expression being induced [1]. This suggests that NO, because it is a potential cause of tissue injury, may be an appropriate target for therapy of contact dermatitis [1].

EXPERIMENTAL METHODS IN THE STUDY OF CONTACT DERMATITIS AND ITS INHIBITORS

While several alternatives to *in vivo* testing of the pharmacological and/or toxicological activity of allergens exist, the *in vitro* techniques are not appropriate for the detection and study of structure-activity relationships in the search for new inhibitors of the contact dermatitis reaction. In this case, even with all its incumbent inconveniences, investigating the *in vivo* reaction is essential.

Still, several *in vitro* methods are worth mentioning. Kimber *et al*. [29], for example, have compiled different *in vitro* models for sensitization and have proposed the possible use of T lymphocyte and dendritic cells to provoke antigen-induced secondary responses, also *in vitro*. In the case of dendritic cells, the changes in cytokine mRNA expression, particularly IL-1β mRNA, are of particular interest since the cytokine IL-1β found in the epidermis of mice is produced exclusively by Langerhans cells and seems to be essential for both the induction of these cells and the acquisition of skin sensitization.

In Vivo Protocols in the Study of Contact Dermatitis

Although guinea-pigs have been the species of choice for the study of different agents and potential inhibitory drugs in allergic contact dermatitis, mice were recently included as a valid animal with which to analyze the potential effects of drugs administered after the allergic reaction has been induced. The guinea pig maximization test, the Buehler assay, and the murine local lymph node assay are all adequate, non clinical methods for detecting the potential substances that produce the hypersensitivity reaction [30]. However, the mouse ear swelling test seems to be an easy way in which to study both the antagonists and the inhibitors of these reactions.

Protocols vary from the mouse ear swelling test [21,31] to the local lymph node assay [30,32]. In the first group of protocols, the researcher not only can identify the potential allergens on the basis of challenge-induced increases in ear thickness in sensitized animals (control group), but also undertake a subsequent study on potential inhibitory agents (sensitized animal plus drug). The inhibition of the reaction can be expressed as the decrease in ear swelling or ear weight as compared to the control group. Other parameters can be measured after analysis of the cut ear; these include the presence of enzymes, mediators, cell infiltration, and tissue damage [33,34].

The guinea pig maximization test is well suited to hazard identification, but provides inadequate information for quantitative risk assessment, whereas the murine local lymph node assay has the unique advantage of providing estimates of the relative skin sensitizing potency [35]. In this latter assay, the skin sensitizing potential of agents is measured by their ability to stimulate proliferative responses in lymph nodes draining the site of topical exposure. Other relevant parameters such as the production of cytokines can also be measured [30].

Agents Used as Inducers of Experimental Contact Dermatitis

While the agents used for inducing the allergic reaction may be different, they can all be used in the aforementioned protocols [8]. Animals are sensitized by first applying a sensitizing agent on the shaved abdomen and then several

days later, the sensitized animals are challenged on one ear by topical application of the same agent. The following contact-delayed hypersensitivity reaction can be quantified by measuring the swelling or by weighing [34].

The dose applied for skin sensitization is usually expressed as the amount of allergen per unit area on the skin, although sometimes the results are expressed in units of mg/kg body weight [36]. This is due to the fact that as an allergy response factor, concentration is less significant than area of contact. That is to say, application of varied concentrations of allergen, for example DNCB, over the same amount of skin area produces similar responses, whereas the same dosage applied over variably large areas leads to various responses – the greater the application area, the greater the response. [37].

Picryl chloride, *p*-phenylenediamine (PPD), DNCB, DNFB, and oxazolone are the agents most commonly used in the study of potential agents of contact hypersensitivity reactions [8,21,33]. Some of them act directly as allergens while others are prohaptens that need metabolic activation to give the corresponding haptens [21].

The administration of DNCB induces both types of cells, Th1 and Tc1 and provokes a minor appearance of Th2-type cells. In the case of this particular agent, the relatively high levels of IFN-γ are derived from both CD4 and CD8 cells, and both types of T-lymphocytes contribute to the immune responses as well as to the patterns of cytokine secretion stimulated by exposure to chemical allergens [8]. The contact of the mouse skin with the allergen not only increases the membrane expression of MHC class II molecules, but also that of CD45 (ICAM-1) and CD86 (a costimulatory molecule expressed by antigen presenting cells), as well as IL-1β production by dendritic cells [21].

Oxazolone is a potent contact-sensitizing agent. The reaction is primarily cell-mediated, so animals with no previous contact with the agent can be rendered sensitive by the transfer of cells, lymphocytes, or splenocytes obtained from sensitized animals [38]. The contact dermatitis induced by oxazolone in mice has been shown to produce swelling with concomitant increased tissue levels of leukotrienes (LTs) and prostaglandins (PGs), whereas corticosteroids have been found to inhibit both the swelling and eicosanoid levels. Interestingly, while cyclooxygenase (COX) inhibitors such as indomethacin reduce PG levels and selective 5-lipoxygenase (LOX) inhibitors reduce LT levels, neither set of inhibitors seems to be able to reduce the inflammation, even when administered together [39]. It has also been demonstrated that the DTH reaction can be inhibited by MDP(Lysyl)GDP, a non-toxic muramyl dipeptide derivative which inhibits the expression of different cytokines, including IL-1β, TNF-α, and iNOS [40].

Application of DNFB induces a hypersensitivity-associated inflammation, which in this case, is a cell-mediated immune reaction [33]. Twenty-four hours after elicitation of contact dermatitis in mice previously sensitized by application of DNFB, the expression of iNOS is greatly increased in MHC class II^+ Langerhans cells. At the protein level, twenty-four hours after elicitation of contact dermatitis, iNOS expression is observed in epidermal Langerhans cells, but not in keratinocytes [41].

NATURAL PRODUCTS AS INDUCERS OF CONTACT DERMATITIS

Antigens or haptens usually have a low molecular weight, 500 Dalton or less, which facilitates their absorption by the skin and their subsequent allergenic activity. Their size is a distinct advantage; if they were larger, they would not be able to pass through the corneal layer [3,42]. Once absorbed, these haptens, which are electrophilic molecules, bind covalently to carrier proteins in the skin [43].

Of the approximately six million chemicals in the environment, roughly 3000 are potential contact allergens [6]. Many organic substances, natural or synthetic, as well as some metals, have the capability to induce dermatitis [44]. Nickel, chromium, mercury, cobalt, and gold are the most common allergenic metals, and acrylates, preservatives, emulsifiers, and dyes can all cause immunological reactions. Moreover, allergic contact dermatitis may also be induced by a wide variety of drugs that trigger specific immune responses following topical exposure. Some reactive chemical functions have been identified from common drugs such as the diphenylketone group of the anti-inflammatory drug ketoprofen, the amide or ester structure of local anesthetics, and the side-chain and thiazolidine ring of β-lactams [23,45]. In addition, drugs used against allergic reactions (e.g. steroids) may produce hypersensitivity reactions. Although steroid-induced hypersensitivity reactions are rare, different cases have been described, but always as heterogeneous entities, with no single uniform mechanism. It thus seems that the mechanisms of this reaction vary depending on the patient [46]. The major problem in this particular case is to recognize the reaction clinically, since when corticosteroids are used to treat eczema, the appearance of a topical contact allergy is considered to be a failure in treatment [7].

Some plants or natural products from plants can enhance the allergic or hypersensitive reactions caused by specific compounds. While type I or immediate hypersensitivity includes urticaria or angioedema, type IV or delayed hypersensitivity reactions manifest themselves as allergic contact dermatitis. A third group of plants contains substances that produce direct toxicity in skin; psoralen-containing plants, for example, induce phototoxic reactions by forming pyridine crosslinks in DNA [47].

Plants have relevance in dermatology for both their adverse and beneficial effects on skin disorders and the skin in general [48]. In this review, we are principally interested in the immune response produced as a reaction against plants or their constituents.

Allergenic Substances of Natural Origin

A natural product can either act directly as an allergen or, in some cases, upon metabolism it can suffer a structural modification, which gives it the capacity to act as an allergen. If the compound does not have the capacity to produce a direct immune response, it must acquire the ability to form a conjugate with proteins so that it can be processed

in this state and presented to the immune system. A second mechanism implicates a direct binding to those peptides associated with major histocompatibility gene products [8]. The effectiveness of skin sensitization is influenced by the potency of the allergen, the amount of substance at the skin surface, and the degree of inflammation induced. The pathological response of contact dermatitis depends on the exposure and potency of the allergen that causes the reaction, as well as the particular susceptibility of each individual [8].

Medicinal Plants that Produce Contact Dermatitis

Plants have been shown to produce allergic contact dermatitis, and while it is not common, in some cases the reaction can be severe. Interestingly, some medicinal plants produce allergic reactions while others are specifically used as anti-inflammatory agents; in some cases they even have anti-allergic properties. An allergenic agent or plant can usually produce contact dermatitis after direct contact, but sensitization occurs only after repeated exposure to a given extract of medicinal plants, food, or spices [47,49,50].

Some of the adverse effects of plants on the skin include irritant contact dermatitis caused mechanically or by chemical irritants found in plant sap, specially from members of the Ranunculaceae, Euphorbiaceae, and Asteraceae (Compositae) plant families, and phytophotodermatitis caused by plants containing furocoumarins, which notably include the members of the Umbelliferae, Rutaceae, and Moraceae plant families. Allergic contact dermatitis is induced primarily by plants from the Asteraceae family, which contain sesquiterpene lactones, but other families containing this class of compounds, specially the Lauraceae, Magnoliaceae, and Jubulaceae families, can also produce allergic reactions. The Anacardiaceae family includes a relevant group of potential plant with allergens, such as *Anacardium occidentale* and *Rhus radicans* or *Toxicodendron radicans* (poison ivy), as well as other species of *Rhus* containing urushiol (Fig. **1**) [7]. However, other species from this family have beneficial effects and can be used in the treatment of skin disorders; thus *Aloe vera* is used to heal wounds and burn injuries, tea tree oil obtained from *Melaleuca alternifolia* ameliorates skin infections such as acne, herpes and scabies, and various compounds are used to fight psoriasis and other inflammatory and immune disorders affecting the skin [7,48].

Still, sometimes even those compounds with proven beneficial effects can cause irritation. *Aloe vera* extract, for example, which is widely employed in pharmacy, phytotherapy, and cosmetics to treat pruritus, burns, dermatitis, and skin dryness, can also produce dermatitis after topical application due to the anthraquinones aloin and emodin, which act as allergens [47].

Another example involves *Boswellia serrata*, a species whose anti-inflammatory properties [51] are due to its content in boswellic acids [52], principally the acetyl-β-boswellic acid [53]. This compound not only inhibits 5-LOX activity by means of a non-redox mechanism [54], but it also has an inhibitory effect on the serine proteases, including human leukocyte elastase [55]. It is thus used against skin disorders in which 5-LOX is over-expressed. However, a case of allergic dermatitis was reported after topical application of incense obtained from this botanical species [47].

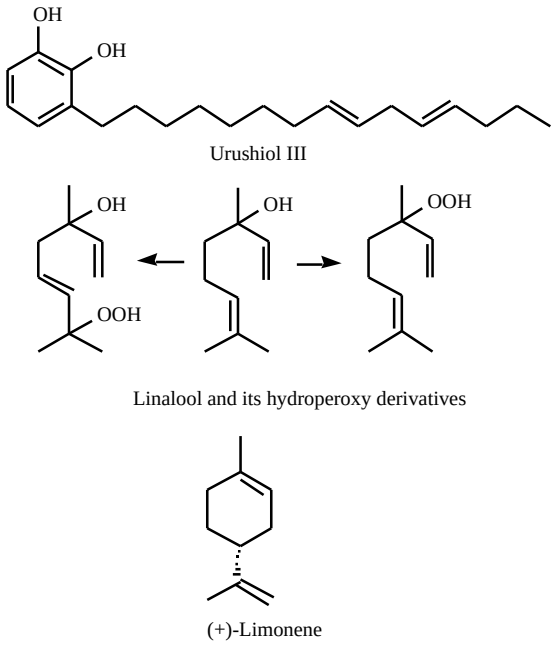

Fig. (1).

Species with potential allergens, such as *trans*-cinnamaldehyde, *trans*-cinnamic alcohol [56,57], geraniol, hydroxycitronellal [57], eugenol, and isoeugenol [11,57], are widely used in perfumery, pharmaceutical products, food spices, folk medicine, and phytotherapy. They are common constituents of species such as *Commiphora myrrha* var. *momol* (myrrh), *Cinnamomum zeylanicum* (cinnamon) and *Syzygium aromaticum* (clove). Fragrances are mixtures of compounds with a low molecular-weight (see above), which may induce allergic contact dermatitis by fragrance-specific $CD4^+$ and $CD8^+$ T lymphocytes [57]. Moreover, in the allergic contact dermatitis produced by cinnamaldehyde, a common constituent of fragrances and spices, both skin absorption and metabolism play a role [56].

Destructive distillation of the wood of *Juniperus oxycedrus* gives the oil of cade, an ingredient in many preparations used in the treatment of psoriasis, but it can sometimes be both an irritant and an allergen [47].

The oil obtained from *Ricinus communis* (Castor oil plant), used in cosmetics and pharmacy, was reported to sometimes produce allergic contact dermatitis after exposure to lipsticks containing this oil [47].

Inula viscosa is a Mediterranean plant used in folk medicine as an anti-inflammatory agent [58], but it can also cause allergic contact dermatitis [59] because of its content in sesquiterpene lactones. Oddly, these lactones have been reported as the anti-inflammatory principles in this and other species [60]. The allergic reaction produced by this species, along with many others from the Asteraceae family, is caused by direct contact with the allergens present in the oleoresin fraction of the leaf stem and flower [61]. The most common allergenic derivatives described in Compositae

plants are the sesquiterpene lactones alantolactone and isolantolactone.

Allergenic Natural Products

As mentioned above, the best known allergenic substances found in plants are sesquiterpene lactones and furanocoumarins. However, other natural products such as flavonoids [62], alkaloids [63-69], and terpenoids other than sesquiterpene lactones [70-74] can cause allergic reactions. It is worth noting that while flavonoids are only weakly antigenic and usually do not induce immune reactions after consumption or therapeutic application, antibodies against flavonoids have been found in human blood [62].

Table **1** provides a list of the most relevant allergenic substances from medicinal plants. The plants listed are all used in medicine, food, or cosmetics. The most common sources of each compound are included in the table.

NATURAL PRODUCTS AS POTENTIAL AGENTS USED AGAINST CONTACT DERMATITIS

The best method of treatment for contact dermatitis is avoiding the responsible allergen. In the case of drugs, the patient should be treated with other active drugs without potential cross-reactions. While cross-reactions are not always evident, if the disorder is diagnosed early and the allergen avoided, the prognosis is often good [43]. Corticosteroids are the more common treatment in the case of contact dermatitis, but they have a high level of collateral and undesirable effects. The search for new potential active agents against contact dermatitis is thus of great interest, and natural products provide an important source for a wide range of potential new agents.

Topical treatment of contact dermatitis with corticosteroids is usually sufficient to ameliorate the allergic and inflammatory reaction; however, when the area involved is greater than 25% of the total body surface or if the reaction is persistent, systemic administration of corticosteroids is necessary [43]. While the mechanism whereby corticosteroids exert their effects has not yet been elucidated, one likely mechanism is the suppression of the effector phase of DTH response by means of either anti-inflammatory properties or effects on sensitized memory T cells [140]. Tacrolimus, pentoxifylline, and vitamin D_3 derivatives are other compounds used to suppress the various phases of allergic contact dermatitis [141].

New possibilities in contact dermatitis therapy include new classes of immunosuppressants, inhibitors of cellular metabolic activity, inhibitors of cell adhesion molecules, targeted skin application of regulatory cytokines, and neutralization of pro-inflammatory cytokines. One therapy that has already proven to be not viable, however, is the possible hyposensitization of patients to contact dermatitis [43].

Plant Extracts

Vegetable fats, specially palm and rapeseed fats, can act as protective agents in cases of irritant contact dermatitis, but have no effect on allergic contact dermatitis. Schliemann-Willers *et al*. [142] demonstrated that oral and topical application of natural fatty acids, specially unsaturated essential linoleic acid, influences epidermal lipid synthesis and contributes to barrier restoration. In contrast, unsaturated non-essential oleic acid tends to enhance the penetration of different irritants through the *stratum corneum*.

Poison ivy is the most common inductor of contact dermatitis in the plant kingdom. One popular remedy used in the mid-western United States is the oral administration of a decoction of jewel weed (*Impatiens biflora* or *I. pallida*), or topical application of its stem sap to the affected area [143]. However, various double-blind, randomized, and controlled clinical trials on human volunteers clinically demonstrated that jewel weed juice is not effective in preventing dermatitis caused by poison ivy [144,145]. Other species used in folk medicine to treat different types of contact dermatitis are plants with tannins such as witch-hazel (*Hamamelis virginiana*), and menthol or camphor [143].

When administered in the effector phase, the aqueous extract from the rhizome of *Smilax glabra* produced a significant reduction of the picryl chloride-induced DTH to almost normal levels from the levels induced by cyclophosphamide with a normalization of the CD4/CD8 ratio. The extract has also been shown to downregulate the function of over-activated macrophages while upregulating the dysfunctional T lymphocytes from rats. Moreover, it significantly inhibited the production of IL-1, TNF, and NO; in contrast, IL-2 production was actually enhanced [146].

Using different experimental protocols, including the picryl chloride-induced contact dermatitis model, Matsuda *et al*. screened several plants used in Japan as anti-allergic remedies. They demonstrated that the methanol extract obtained from dried rhizomes of *Alisma orientale* reduced the induction phase, most likely by a mechanism in which the antigen blocks the sensitization of the T-cells. Interestingly, the extract had no effect on the effector phase [147]. In contrast, the 70% ethanol extract from the dried fruits of *Kochia scoparia* only showed significant activity against the swelling produced during the effector phase, having no effect on the induction phase [148]. These researchers went on to isolate and study various terpenes from the active extract and found that only the monodesmosidic saponin momordin Ic had an inhibitory effect on the effector phase of the contact dermatitis induced by picryl chloride [148].

Cuéllar *et al*. [149] screened twelve anti-inflammatory medicinal plants used in traditional medicine against skin diseases, concluding that the hydroethanolic extract of *Scrophularia auriculata*, *Santolina chamecyparissus* and *Ranunculus sceleratus* significantly diminished the DTH reaction induced by oxazolone and reduced the inflammatory response during the first 102 h after the challenge. Other plant extracts, including *Codonopsis pilosula*, *Forsythia suspensa*, and *Polygonum multiflorum*, principally inhibited the initial reaction, but had no effect on the inflammatory process, whereas *Asphodelus fistulosus* gave the best effect by the end of the experiment. In a complementary study, the same authors [150] demonstrated the activity of some Asian medicinal plants such as *Phellodendron amurense* and *Scutellaria baicalensis*, but in this case the activity was less than that of the abovementioned species. Moreover, the

Table 1. Allergens from Medicinal Plants

Alkaloids	**Plant Source**	**Reference**
Atropine	*Atropa belladona*	64,66
Pilocarpine	*Pilocarpus* sp.	65
Quinine	*Cinchona* sp.	68
Thebaine, codeine	*Papaver somniferum*	69
Terpenoids	**Plant Source**	**Reference**
Abietic acid	*Pinus* sp.	75
Alantolactone	*Inula helenium*, *Frullania* sp. *Chrysanthemum* sp.	76-79
Artesunate	*Artemisia annua*	80
Asiaticoside, asiatic and madecassic acids	*Centella asiatica*	81,82
Carvone	*Carum carvi*	83-85
Citral	*Citrus* sp.	86
Coleus extract	*Coleus blomi*	87
β-Cyclocostunolide	Costus resinoid	88
Dehydroabietic acid (peroxides / epoxides)	Rosin (colophony)	104-106
Eucalyptol	*Eucaliptus* sp.	89
Farnesol	*Cymbopogon* sp., others	90,91
Geraniol	*Origanum mejorana*, others	92,93,94
(+)-Limonene	*Citrus* sp.	92,95,96
Limonene, α-pinene, phellandrene	*Melaleuca alternifolia*	97
Linalool	*Ocimum basilicum, Coriandrum sativum*, others	74,98
Menthol	Peppermint, *Mentha* sp.	99,100
Myrrh	*Commiphora* sp.	101,102
Parthenolide	*Tanacetum parthenium*	103
Polygodial	*Polygonum* sp., *Drymis* sp.	107
Sesquiterpenes	Liverworts, lichens, mosses	108
Sesquiterpenes	*Parthenium hysterophorus*	109
Sesquiterpene lactones	*Pyrethrum* sp.	110
Sesquiterpene lactones	*Arnica montana*	110
Sesquiterpene lactones	Peruvian Lily (Alstromeria)	111
Sesquiterpene lactones. Alantolactone, dehydrocostuslactone and costunolide	Compositae sp.	61,112,113
Thymol	*Thymus* sp.	72
Ylang-ylang oil	*Cananga odorata*	114
Phenolics	**Plant Source**	**Reference**
Anethol	*Illicium verum* *Pimpinella anisum*	115
Atranorin	Lichens	116,117

(Table 1) contd…..

Catechols	*Greviella robusta*	118
Cinnamon (oil)	*Cinnamomum verum*	119
Cinnamic aldehide	*Cinnamomum verum*	94
Cinnamic derivatives, resorcinol monobenzoate, coniferyl benzoate, coniferyl alcohol, benzyl alcohol, benzoic acid	Balsam of Peru (*Myroxylon balsamum*)	120
Curcumin	Turmeric	121
Eugenol	Clove (essential oil)	73,119,122
Ginkgolic acids	*Ginkgo biloba* (fruits)	123
Isoeugenol	*Cananga odorata*, others	89,94,124
Litreol (3-pentadecyl (10-enyl) catechol) 3- pentadecylcatechol 3- pentadecenylcatechol 3- heptadecenylcatechol 3- heptadecdienylcatechol	*Lithraea caustica* *Lithraea molleoides* *Lithraea brasiliensis*	125-127
3-heptadecylcatechol	Poison oak urushiol oil	128
Resorcinols	*Anacardium occidentale* *Magnifera indica*	129-132
Oak moss resin	*Evernia prunastri*	94,133
Tertiaery-butylhydroquinone (TBHQ)	Vegetable hydraulic oil	134
Urushiol	Poison ivy (*Rhus radicans*) poison oak (*R. diversiloba*) poison sumac (*R. vernix*) (*Rhus* or *Toxicodendron*)	135-138
Usnic acid	Lichens	139

ethanol extract from the fungus *Poria cocos* inhibited the inflammatory response to the DTH reaction induced by oxazolone, but did not affect the induction phase. Interestingly, the individual compounds isolated from this species did not modify the inflammation [151].

Haplophyllum hispanicum is a medicinal plant used by dermatologists against irritant and inflammatory dermatitis. One well-known property of this plant is its photosensitizing ability; for this reason, it is commonly used in phytomedicine to treat vitiligo and other alterations in skin pigmentation. Prieto *et al*. [152] studied the effect of the ethanol extract of this species on different experimental models of inflammation, including the oxazolone-induced DTH reaction on the ears of mice. These researchers observed that whereas the extract has anti-inflammatory effects on the TPA-induced mouse ear edema, the same extract increased the inflammation induced by oxazolone. The anti-inflammatory compounds isolated were aryl naphtalide lignans, such as diphyllin acetyl apioside and tuberculatin, while the sensitizers would be the coumarins detected in the fresh plant.

Another botanical species with compounds potentially active against contact dermatitis is the Asteraceae *Phagnalon rupestre* [153]. When administered after challenge, the methanol extract of this species reduced the edema induced by DNFB as well as those caused by sheep red blood cells (SRBC). The fractionation of the methanol extract with solvent of increased polarity and a subsequent assay using the DNFB test demonstrated that the active compounds are principally located in the ethyl acetate extract from which seven compounds were isolated and investigated against DNFB-induced contact hypersensitivity. Four of these compounds were found to inhibit the DTH reactions.

Phenolics

From *Phagnalon rupestre*, Góngora *et al*. [153] isolated one flavonoid: luteolin-7-*O*-β-glucoside ; two hydroquinone glycosides: 1-*O*-β-glucopyranosyl-2-(3'-hydroxymethyl-3'-methylallyl) hydroquinone and 1-*O*-(4"-*O*-caffeoyl)-β-glucopyranosyl-2-(3',3'-dimethylallyl) hydroquinone; and one dicaffeoylquinic acid derivative: 3,5-di-*O*-caffeoylquinic acid methyl ester (Fig. **2**). They reduced the DNFB-induced contact hypersensivity by approximately 49%, 47%, 45%, and 43%, respectively (induction phase), and by 79%, 73%, 63%, and 55%, respectively (effector phase). However, two structurally related derivatives: 1-*O*-β-glucopyranosyl-2-(3',3'-dimethylallyl) hydroquinone and 4,5-di-*O*-caffeoylquinic acid, only inhibited the inflammatory reaction without affecting the induction phase. In contrast, 3,5-di-*O*-

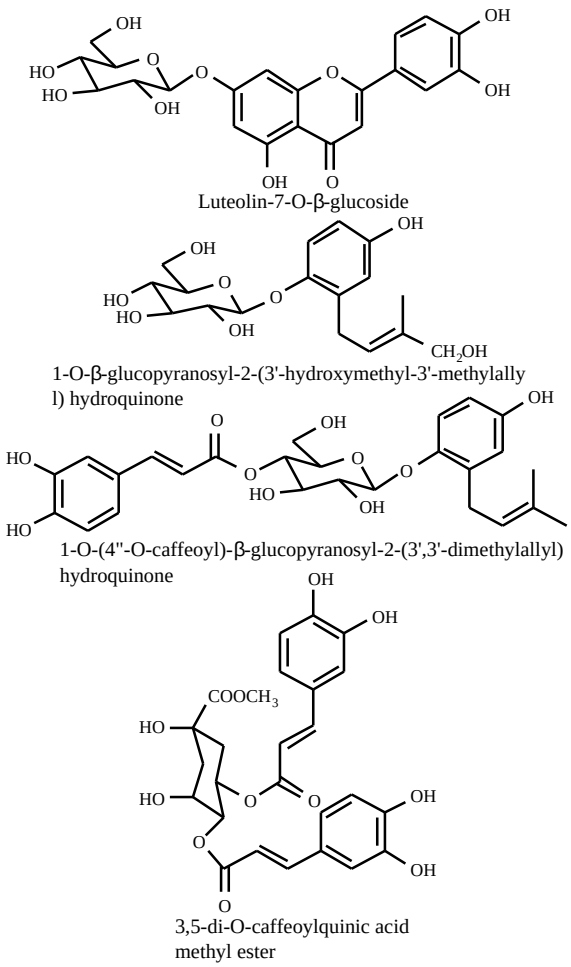

Fig. (2).

caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid were inactive in both induction and effector phases. Unfortunately, this study did not establish a relationship between chemical structure and pharmacological activity, but the potential of this class of compounds as possible inhibitors of the DTH reaction should not be dismissed.

Arbutin (Fig. **3**), a glucosyl-hidroquinone derivative, is the antiseptic principle from bearberry leaf (*Arctostaphyllos uva-ursi*). This compound was studied as a potential inhibitor of DTH reactions as well as for its possible cross-reaction with corticosteroids. When administered orally immediately before and 16 h after challenge, arbutin showed no activity, but when it was administered 24 h after the application of the allergen, it decreased the ear swelling induced by picryl chloride in mice. Moreover, it did so without causing a decrease in thymus or spleen weight, a common side effect of prednisolone or dexamethasone. In addition, the effect of these corticosteroids was found to be stronger when they were administered together with arbutin [154].

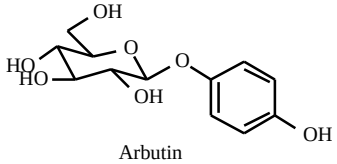

Fig. (3).

Flavonoids

In addition to the luteolin-7-*O*-β-glucoside mentioned above, other flavonoids have been studied as potential inhibitors of DTH reactions (Fig. **4**). For example, apigenin showed anti-inflammatory activity against the DTH reaction in mice. On the other hand, while mauritanin and myricitin were shown to reduce the DTH reaction to DNFB in mice, neither compound had any effect on the DTH reaction to SRBC.

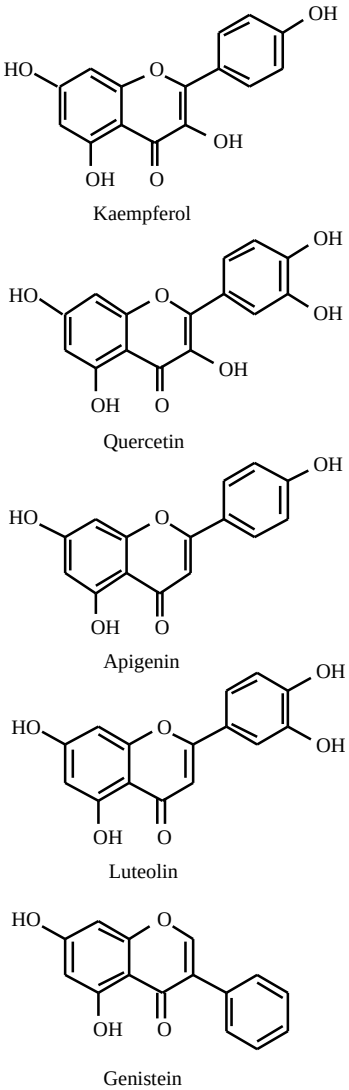

Fig. (4).

As explained above, iNOS expression is induced in contact dermatitis and during the first phase after elicitation, NO produces vasodilatation and attraction of neutrophils to finally cause inflammatory responses in the implicated tissues. Some flavonoids such as kaempferol (IC_{50} 5.7 μM) and apigenin (IC_{50} 6.86 μM) inhibit iNOS induction at the level of iNOS gene transcription, but they do not actually modify NOS activity [155]. Other flavonoids with similar

properties are quercetin and its glycoside rutin, primuletin, catechin, hesperetin, hesperidin, and naringenin, but the potency of all these compounds was clearly less than that of the previously mentioned flavonoids (IC_{50} between 63 and 191.2 μM).

Luteolin is a known inhibitor of protein kinase C (PKC), which is implicated in the migration of Langerhans cells from the epidermis [156]. Moreover, luteolin inhibits the expression of ICAM-1 [157], which is essential for the infiltration of leukocytes into peripheral tissues [1].

Apigenin exhibits anti-inflammatory activity in both carrageenan-induced rat paw edema and the DTH reaction in mice. Together with other related flavonoids it blocks cytokine-induced ICAM-1, VCAM-1, and E-selectin expression on human endothelial cells. Apigenin also modifies both adhesion protein expression and adhesion protein upregulation at the transcriptional level. It has also been shown to inhibit IL-1α-induced PG synthesis and TNF-α-induced IL-6 and IL-8 production, but not TNF-α-induced nuclear translocation of nuclear factor-κB (NF-κB). These data suggest a possible effect of apigenin as a general inhibitor of cytokine-induced gene expression; it is most likely this mechanism that is involved in the anti-inflammatory and anti-allergic properties of this flavonoid and others of similar chemical structure [157].

Genistein is a known phytoestrogen present in soy (*Glycine max*) and other common plants. Phytoestrogens bind to the classical estrogen receptor ERα; more interesting, however, is the fact that genistein binds to the recently discovered ERβ. This could explain some of the pharmacological properties of this flavonoid, since not only it has been demonstrated that along with macrophages, both $CD4^+$ and $CD8^+$ T cells can express estrogen receptors, but also that estrogens can decrease the cell-mediated immunity in mice. Genistein is able to decrease the DTH response induced by 4-hydroxy-3-nitrophenyl acetylsuccinimide, but the fact that this effect is not abolished by the ER antagonists indicates that the effects of genistein on cell-mediated immunity involve both ER-mediated and non-ER-mediated components. The measurements of $CD4^+$ and $CD8^+$ T cells demonstrate that both cells are reduced following treatment with genistein; this, in turn, could be one of the causes of the observed reduction of the DTH response [158].

Some indirect mechanisms implicate flavonoids in the modulation of the immune response. These include the interference with both protein phosphokinases and transport ATPases (implicated in the regulation of cell homeostasis), the inhibition of IL-5 activity (chemotactic), lysosomal secretion, arachidonic acid metabolism and Ca^{2+} fluxes [62]. Other potential effects and mechanisms have been reviewed by Middleton *et al*. [159] and Havsteen [62].

The flavonoids from green tea, quercetin and chrysin, are specially interesting. When administered in the diet, these compounds prevented the UV-induced suppression of contact hypersensitivity to picryl chloride in mice. In the skin of mice treated concomitantly with these flavonoids and UV radiation, the number of Langerhans cells were similar to the control, whereas the number of Langerhans cells in mice treated with UV radiation alone decreased. The authors of this study hypothesize that quercetin and chrysin, along with other similar flavonoids, could play a role in the prevention of UV-induced immunosuppression [160].

Terpenoids

Sesquiterpene lactones are a group of natural products widely distributed throughout the plant kingdom and specially common in the Asteraceae family. Some of these compounds have been reported to be haptens as well as anti-inflammatory principles of medicinal plants with effects against DTH reactions [161]. Comparative studies of a large number of compounds have demonstrated that the presence of an α-methylene-γ-lactone in the chemical structure of the compounds is required for them to exhibit anti-inflammatory activity. Moreover, this same chemical group is essential for the inhibition of iNOS dependent NO synthesis. Other chemical groups in the structure, including α,β-unsubstituted cyclopentenone, seem to be less relevant [162]. In an additional work, Hall *et al*. demonstrated that sesquiterpene lactones inhibit cell migration, lysosomal rupture and enzymatic activity, along with PG synthesis, which was linked to elevated cyclic adenosine monophosphate (cAMP) levels [163].

Several sesquiterpene lactones with pseudoguaianolide structure were tested as potential inhibitors of DTH induced by oxazolone (Fig. **5**). While the derivatives with a C-4 carbonyl group (tetraneurin A and parthenin) reduced both the induction phase and neutrophil infiltration, two other acetylated compounds, hysterin and ambrosanolide, showed no activity in the induction phase and did not affect cell infiltration. The latter two compounds did, however, clearly inhibit the effector phase. A fifth compound, confertdiolide, with a different substitution partner, showed effects similar to those of these latter two compounds [164].

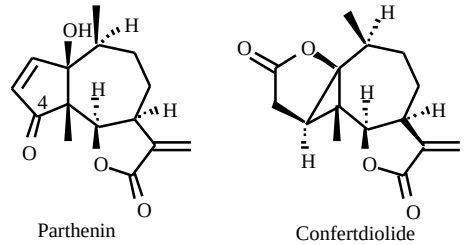

Fig. (5).

Complementary studies on the mechanism of action of sesquiterpene lactones as anti-inflammatory and anti-allergic agents implicated the NF-κB, a central mediator of the immune response, and the consequent modification of different peptidic mediators or pro-inflammatory enzymes. For example, diversiflorine from *Tithonia diversiflora* inhibits the activation of NF-κB by alkylation of cysteine residues, which in turn inhibits the synthesis of cytokines and chemokines [165]; cynaropicrin from *Saussurea lappa* inhibits TNF-α production [166,167], lymphocyte proliferation, and NO accumulation by means of a mechanism in which the conjugation of the compound with the sulphydryl groups of the target proteins is implicated [166]; parthenolide (Fig. **6**) from *Tanacetum parthenium* (feverfew) inhibits the activation of NF-κB by preventing the

degradation of both IκB-α and IκB-β [168], while simultaneously suppressing iNOS promoter activity by inhibiting the iNOS gene [169]; nepalolide from *Carpesium nepalense* suppresses the expression of iNOS by blocking IκB degradation [170]; the potential allergenic costunolide from *Magnolia grandiflora* and the dehydrocostus lactone from *Saussurea lappa* both modify Tc lymphocyte functions [166], decrease TNF-α levels [171] and inhibit NO production by targeting IκB phosphorylation and degradation to inhibit NF-κB, thereby downregulating iNOS expression [172]. However, the Merfort's work with six different sesquiterpene lactones demonstrated that all of them inhibit the NF-κB signaling cascade by targeting the active heterodimer directly [173]. In this sense, helenalin (Fig. **6**) from *Arnica montana* was found to be a selective inhibitor of NF-κB [174], inhibiting not only the proinflammatory gene expression, but also strongly downregulating the mRNA expression in $CD4^+$ cells [175]. Helenalin inhibited neither IκB degradation nor NF-κB translocation, but rather modified NF-κB directly by means of a selective alkylation of the p65 subunit of NF-κB [173].

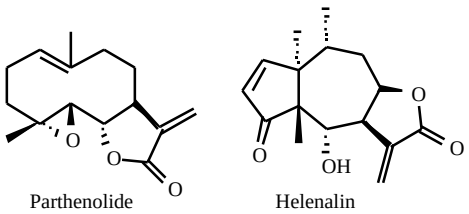

Fig. (6).

The diterpene triptonide (Fig. **7**), obtained from *Tryptergyum wilfordii* inhibited the DTH reaction to DNFB in the skin of mice. The mechanism was related to the reduction of the helper T cell to suppressor T cell ratio as well as to the induction of suppressor T cells without modifying the IL-2 secretion [176]. Still other diterpenoids, including andalusol from *Sideritis foetens*, are able to exert their effect through the inactivation of NF-κB and the consequent inhibition of iNOS expression [177].

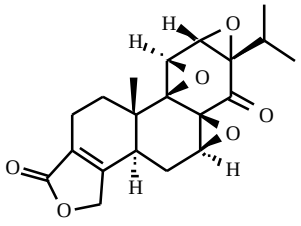

Fig. (7).

Tripterine (celastrol), a triterpene isolated from *Tryptergyum wilfordii* (Fig. **8**), inhibited the DNCB-induced DTH reaction in the skin of mice [178]. Recent studies demonstrated tripterine' ability to inhibit the production of IL-1β, IL-1α, and TNF-α from lipopolysaccharide (LPS)-stimulated human monocytes with IC_{50} values of 40 nM [179], 40 nM, and 210 nM [180], respectively. This finding is interesting for several reasons, not least of which is the fact that IL-1β is essential for the initiation of cutaneous immune responses. In addition, not only is the upregulation of IL-1β by Langerhans cells relevant in the early stages of skin sensitization, but this same cytokine, together with TNF-α, also plays a relevant role in Langerhans cell mobilization and the development of contact dermatitis.

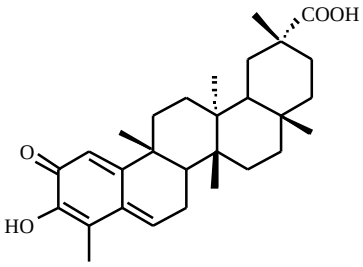

Fig. (8).

In a comparative study between the anti-allergic activity of bryonolic acid (Fig. **9**) from *Luffa cylindrica* and glycyrrhetinic acid from licorice (*Glycyrrhiza glabra*) on contact hypersensitivity induced by picryl chloride in mice, bryonolic acid was found to suppress ear swelling in a dose-dependent fashion over time. Thus, at a dose of 300 mg/kg, swelling was reduced by 90%, 93%, and 98% after 9, 24 and 48 h, respectively. This is a stark contrast to the group treated with glycyrrhetinic acid, in which 8 of 10 mice died within 24 h after intraperitoneal administration of the same dose. The lethal dose (LD)-50 for bryonolic acid was much higher, about 10 g/kg [181]. Both compounds are triterpene derivatives, but while bryonolic acid is a friedoolean-type compound, glycyrrhetinic acid is of the oleanan-type. This fact accounts for both the activity and the toxicity, as well as for the effectiveness of bryonolic acid in DTH reactions.

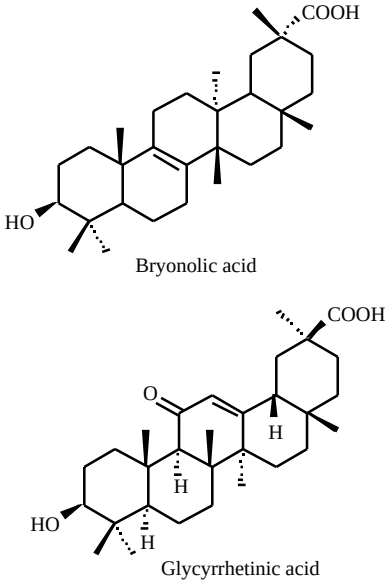

Fig. (9).

From *Scrophularia auriculata*, Giner *et al*. [182] isolated four glycoterpenoids, two saponins and two iridoids. When these isolated compounds were assayed against the DTH reaction induced by oxazolone, only the iridoids were active, specially scrovalentinoside (Fig. **10**), which reduced the

inflammatory response both in the early stage and throughout the inflammatory process, and decreased cell infiltration as well. When compared with the control group after challenge, the ear showed a mild inflammatory lesion, discrete fibrosis and hyperkeratosis, reduction of epithelium thickness, and suppressed infiltration of macrophages and neutrophils.

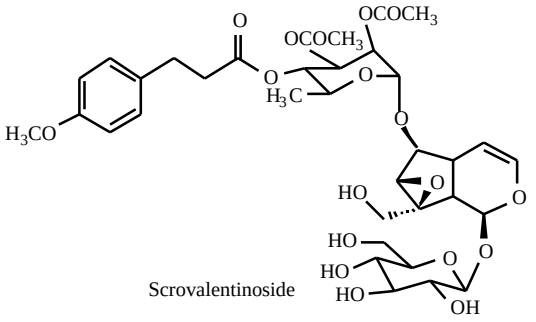

Fig. (10).

Alkaloids

Alkaloids that modify the immune system can thus be used to prevent DTH reactions. Two examples of such compounds are colchicine and vinblastine, which act on cellular microtubules to interfere with the transport of proteins from the protoplasm to the cell surface, thereby preventing the appearance of the human leukocyte-associated antigen (HLA)-DR [183].

Kasahara *et al*. studied the suppressive effect of morphine on DTH reactions caused by exposure to trinitrochlorobenzene (TNCB) [184]. Subcutaneous and intracisternal administration of morphine in mice just before TNCB application suppressed the maximal extent of ear swelling at 24 h after challenge in intact mice, although the effect was not significant in hypophysectomized mice. The pharmacological effect was suppressed by intracisternal application of naloxone. These results suggest that the activation of opioid receptor-mediated pathways in the brain after the application of morphine is relevant in the suppression of TNCB-induced DTH reactions [184].

Alkaloids from *Tylophora indica* inhibited the contact sensitivity to DNFB when administered either before or after challenge. Moreover, when lymphocytes from sensitized mice were treated *in vitro* with a mixture of alkaloids and transferred into naive syngeneic hosts, they suppressed the transfer of the DTH response in treated mice as compared to that of the control group [185]. When the mixture of alkaloids was studied *in vitro* against concanavallin A-stimulated splenocytes, the alkaloids inhibited IL-2 production at the inhibitory concentration or higher, but increased it at lower concentrations. At these low concentrations, the lymphoproliferation increases because of the enhanced IL-2 production, whereas the inhibition of proliferation at higher doses is due to the inhibition of IL-2 production along with the parallel activation of macrophages, which have a cytostatic effect [186].

When administered intraperitoneally, indirubin (Fig. **11**), an isomer of indigo isolated from *Polygonum tinctorium*, inhibited the ear swelling caused by TNCB-induced DTH reactions. Moreover, while indirubin inhibited the IFN-γ production by elicited mouse lymphocytes, it did not affect IL-4 production. In contrast, dexamethasone inhibits both IFN-γ and IL-4 production. These results suggest that indirubin may tip the T cell type balance in favor of T helper type 2 whereas the inhibition of the immune response in the case of dexamethasone is non-selective [187].

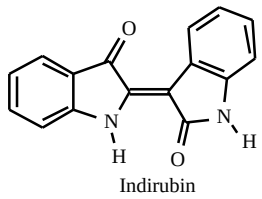

Fig. (11).

Dehydrocorydaline (Fig. **12**) isolated from *Corydalis turtschaninovii* was screened for activity against different types of allergic reactions and was found to inhibit the induction phase of picryl chloride-induced contact dermatitis in mice at a concentration of 125 μmol/kg. In contrast, doses of 500 μmol/kg were necessary to inhibit the effector phase. The authors hypothesize that dehydrocorydaline may influence the activation of lymphocytes or the release of chemical mediators such as lymphokines [188].

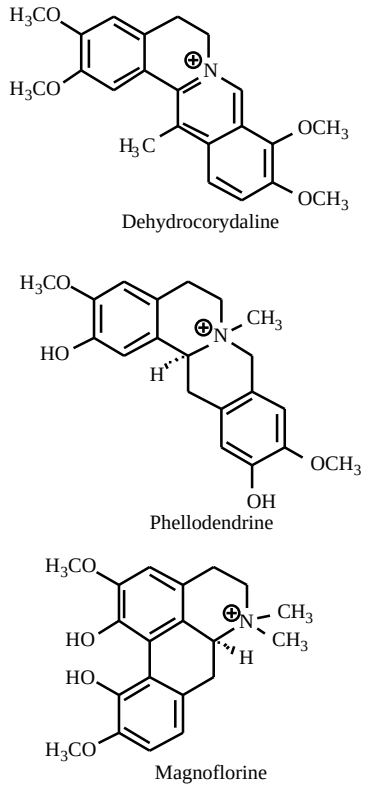

Fig. (12).

In a study on the suppression of cellular and humoral immune responses of principles isolated from *Phellodendron amurense*, Mori *et al*. demonstrated that magnoflorine hydroxide and phellodendrine acetate (Fig. **12**) at 10 and 20 mg/kg significantly suppressed the induction phase of picryl

chloride-induced DTH reactions but did not affect the reaction in the effector phase [189]. The fact that neither compound exhibited anti-inflammatory activity indicates that these substances specifically suppress the cellular immune response without affecting the humoral immune response [190].

Other Compounds

Antioxidants such as ascorbic acid or α-tocopherol may inhibit the allergic reaction after epidermal application because they can trap the free radicals generated by potential pro-allergenic substances. (+)-Limonene from citrus can undergo an auto-oxidation process to give limonene hydroperoxydes (Fig. **1**), which are potent allergenic agents [95]. Application of anti-oxidant compounds has been found to reduce the DTH response in animals both in the sensitization and effector phases. This protective effect could be of interest in humans, but only as a possible treatment for patients exposed to haptens that yield antigens via a radical mechanism. For haptens that form antigens by means of nucleophilic-electrophilic reactions, this effect has no benefit [191].

Fucoidin (*Fucus* sp.) is an algal sulphated fucose polymer that inhibits the contact hypersensitivity induced by TNCB in mice in both the induction and the effector phases. Moreover, fucoidin reduces cell infiltration and edema formation in sensitized mice before and after sensitization. The pharmacological effect is related to the selective inhibition of the CD62L (L-selectin) function, which plays a relevant role in the cutaneous DTH reaction. By preventing the rolling of lymphocytes and monocytes on endothelium by the inhibitors of CD62L functions, the reduction of the DTH reaction is actively provoked. Interestingly, this inhibitory effect is specific for fucoidin, since the monosaccharide fucose and other fucoidin related molecules such as chondroitin 4-sulphate and chondroitin 6-sulphate, showed no inhibitory effects in the experimental model used [192].

CONCLUSIONS

Contact dermatitis can be induced by plants and the natural products obtained from them, but these same products can simultaneously diminish and even abolish the hypersensitivity caused by other plants or chemicals. From the plants extracts studied, some, such as *Scrophularia auriculata* and *Phagnalon rupestre*, which demonstrated their effects in experimental models of DTH reactions induced by different allergens, are highly interesting. Moreover, in both cases the active principles were isolated, identified and their pharmacological effects were established. This research, and subsequent determination of the mechanisms of action involved, could lead to the discovery of new anti-allergic agents.

Other researchers have studied different groups of active compounds. Some of these, such as colchicine, are known immunosuppressive agents, others, such as morphine, are well-established analgesics. However, the groups of natural products with the most effective inhibitory properties against contact dermatitis are the phenolics and terpenoids. Many of these compounds act by means of a non-specific mechanism (e.g. antioxidants), but may also act via specific mechanisms such as the inhibition of the mediators implicated in the immune response.

ABBREVIATIONS

APC	=	Antigen-presenting cells
COX	=	Cyclooxygenase
DNCB	=	Dinitrochlorobenzene
DNFB	=	Dinitrofluorobenzene
DTH	=	Delayed-type hypersensitivity
GM-CSF	=	Granulocyte/macrophage colony-stimulating factor
HLA	=	Human leukocyte-associated antigen
ICAM	=	Intercellular adhesion molecule
IκB	=	Family of inhibitory proteins of NF-κB
IL	=	Interleukin
IFN	=	Interferon
LOX	=	Lipoxygenase
LPS	=	Lipopolysaccharide
LT	=	Leukotriene
MHC	=	Major histocompatibility complex
NF-κB	=	Nuclear factor-κB
NK	=	Natural killer
NO	=	Nitric oxide
NOS	=	Nitric oxide synthase
iNOS	=	Inducible nitric oxide synthase
PG	=	Prostaglandin
SRBC	=	Sheep red blood cells
TNCB	=	Trinitrochlorobenzene
TNF	=	Tumor necrosis factor
VCAM	=	Vascular cells adhesion molecule

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Received: January 14, 2004 Accepted: August 14, 2004