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Anti-Inflammatory Compounds of Plant Origin. Part II. Modulation of Pro-Inflammatory Cytokines, Chemokines and Adhesion Molecules

Abstract

It has been widely shown that many plant-derived compounds present significant anti-inflammatory effects. For this reason, they represent potential molecules for the development of new drugs, especially designed for the treatment and/or control of chronic inflammatory states such as rheumatism, asthma, inflammatory bowel diseases, atherosclerosis, etc. This review focuses on the naturally-occurring compounds with anti-inflammatory properties and attempts to correlate their actions with the modulation of cytokines and associated intracellular signaling pathways; it continues the review published in the November, 2003 issue of *Planta Medica*.

Key words

Medicinal plants · plant constituents · inflammation · cytokines · chemokines · adhesion molecules

Abbreviations

AP-1: activator protein-1
CCR1: chemokine receptor 1
CINC-1: cytokine-induced neutrophil chemoattractant 1
COX: cyclooxygenase

EGCG: (–)-epigallocatechin gallate
ELAM-1: endothelial-leukocyte adhesion molecule-1
ERK: extracellular signal-regulated kinase
GRO: growth-related oncogene
HUVEC: human umbilical vein endothelial cells
ICAM-1: intercellular adhesion molecule-1
IFN: interferon
IL: interleukin
iNOS: inducible nitric oxide synthase
IRA: the natural interleukin receptor activation
JAK: janus kinase
JNK: c-Jun NH₂-terminal kinase
LPS: lipopolysaccharide
MAPK: mitogen-activated protein kinases
MCP: monocyte chemotactic protein
MHC: major histocompatibility complex
MIP: macrophage inflammatory protein
MMP: matrix metalloproteinases
MPO: myeloperoxidase
NF- κ B: nuclear factor kappa B
NO: nitric oxide
PAF: platelet aggregation factor
PGEE: prostaglandin
PK: protein kinase

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PMA/TPA: phorbol myristate acetate
 RANTES: regulated upon activation normal T-cell expressed
 and secreted
 TGF- β : transforming growth factor- β

TNF α : tumour necrosis factor
 VCAM-1: vascular cell adhesion molecule-1

Introduction

The inflammatory process may be defined as a sequence of events that occurs in response to noxious stimuli, trauma or infection. These responses are orchestrated by a highly modulated interaction between mediators of inflammation and inflammatory cells [1]. Cytokines represent a group of multifunctional substances that are involved in many steps of the inflammatory response. Until now, more than 100 members of the cytokine family and their specific receptors have been identified [2], [3], [4]. Generally, cytokines can be classified as pro- or anti-inflammatory, depending on the way they influence inflammation. In a more simplified view, pro-inflammatory cytokines (e.g., IL-1 β , TNF α , IL-6 and IL-18) seem to be involved in the initiation and amplification of the inflammatory process, whereas the anti-inflammatory cytokines (e.g., IL-10, TGF- β and IRA) negatively modulate these events [5], [6].

Cytokines are produced by both resident and migrating cells, such as mast cells, macrophages and neutrophils and, after release; they can act either locally or systemically. Due to their redundant and pleiotropic actions, cytokines form a network in which one cytokine can induce its own production or even the secondary generation of other cytokines. In addition, it has been widely shown that most cytokine actions involve the activation of transcription factors (e.g., NF- κ B and AP-1) and protein kinases (e.g., MAPK and PKC) that, in turn, regulate the expression of many target genes indispensable to the maintenance of the inflammatory state [3], [7]. For instance, cytokines may be responsible for the induction of several enzymes (e.g., iNOS and COX-2), receptors (PAF receptor, IL-2 receptor) and adhesion molecules (E-selectin, α - and β -integrins, ICAM-1, VCAM-1) [3], [8], [9].

A distinct group of cytokines, denoted chemokines (including IL-8, eotaxin, GRO, CINC-1 and RANTES) have the ability to chemoattract and activate leukocytes at the site of inflammation [10], [11]. Chemokine effects are mediated via interaction with G protein-coupled receptors. Binding of chemokines to their specific receptors allows rolling leukocytes to become firmly adherent and able to transmigrate to the target tissue, a process largely dependent on the activation of adhesion molecules, mainly integrins [10], [12].

Recent evidence has indicated that cytokines (and chemokines), as well as their receptors, are involved in the pathophysiology of many inflammatory diseases, including sepsis, rheumatoid arthritis, atherosclerosis and asthma. These pathological states seem to be linked to an imbalance of the cytokine network and to the excessive recruitment of leukocytes to the inflammatory sites [3], [13], [15]. Because of this, the cytokine system constitutes a very interesting and promising target for the development of clinically relevant anti-inflammatory drugs. In fact, some specific cytokine modulators have been introduced into clinical

practice during the last few years. Despite the effectiveness described for these drugs, their use is associated with some side effects [15], [16], [17] and, most important, with high cost. In this context, the identification of small molecule plant-derived compounds able to selectively interfere with the production and/or function of cytokines would constitute an important alternative for the treatment of many inflammatory diseases.

In the second part of this review we will focus on the recent contributions to the identification of naturally-occurring compounds derived from plants as potential modulators of the cytokine network and related cell migration process.

Phenolic Compounds

A substantial body of evidence obtained from both *in vivo* and *in vitro* studies supports the concept that various plant-derived compounds with anti-inflammatory properties exert their effects through the modulation of the cytokine system (for recent review see [18]). For instance, flavonoids, a class of compounds widely distributed throughout the plant kingdom, possess interesting anti-inflammatory actions [19], [20], [21]. Very recently, Xagorari et al. [22] have reported that luteolin (**1**) (IC₅₀ < 1 μ M), quercetin (**2**) (IC₅₀ 1 μ M), luteolin 7-glucoside (approximate IC₅₀ 50 μ M) and the isoflavonoid genistein (IC₅₀ 5 μ M) (**3**) inhibited LPS-stimulated TNF α and interleukin-6 release in RAW 264.7 macrophages, whereas eriodictyol and hesperetin only inhibited TNF α release (approximate IC₅₀ value of 50 μ M). Luteolin also inhibited the production of TNF α *in vivo* and was capable of decreasing both PMA and oxazolone-induced allergic ear oedema [23]. Luteolin significantly reduced LPS-stimulated ICAM-1 expression in the liver of LPS (*Salmonella enteritidis*)-treated mice [24]. In a recent study, Das et al. [25] demonstrated that chronic administration of luteolin significantly attenuated ovalbumin-induced airway bronchoconstriction and bronchial hyperreactivity. Moreover, the same treatment with luteolin was capable of reducing the levels of both IL-4 and IL-5, whereas it induced an increase in IFN γ in the bronchoalveolar lavage fluid of sensitised mice [25]. These authors have suggested that luteolin could be used as a lead molecule to identify effective therapies for the treatment of asthma.

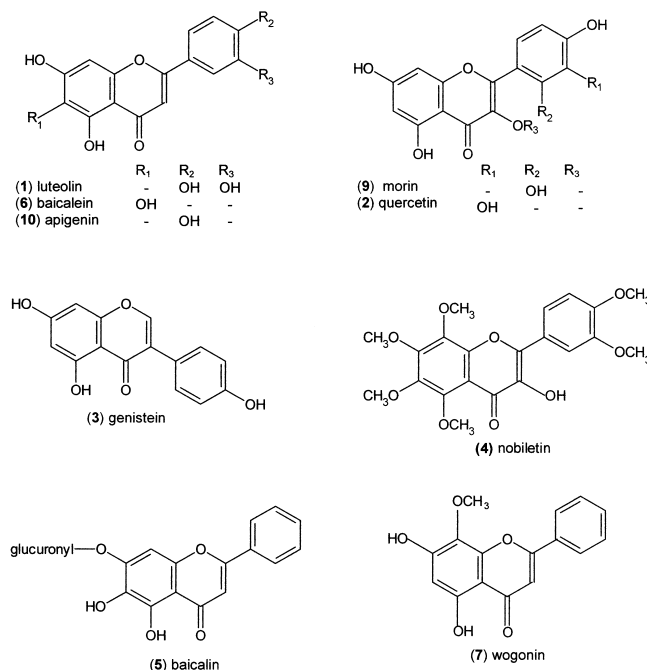
Manna et al. [26] demonstrated that silymarin, a mixture of bioactive flavonoids isolated from *Silybum marianum* L. (Asteraceae) was able to prevent, in a concentration-dependent manner, TNF α -induced NF- κ B activation in human histiocytic lymphoma U-937 cells. Johnson et al. [27] also reported the effects of silymarin on the thymic lymphocyte population in mice. Intraperitoneal administration of silymarin (10 to 250 mg/kg, once a day, for five days) resulted in the augmentation of CD4⁺ and CD8⁺ thymic lymphocyte populations, by a mechanism involving an increase in c-myc expression. In addition, silymarin significantly

decreased the expression of IL-2 and IL-4, without affecting MHC II expression in mouse lymphocytes [27].

It has been demonstrated that a citrus polymethoxyflavonoid, nobiletin (**4**) (5,6,7,8,3',4'-hexamethoxyflavone) effectively inhibits the production of PGE₂ and proMMP-9 in rabbit synovial fibroblasts [28]. More recently, Lin et al. [29] have shown that nobiletin (IC₅₀ < 4 μM) suppressed IL-1β-induced production of PGE₂ in human synovial fibroblasts cells and decreased the expression of IL-1α, IL-1β, TNFα and IL-6 mRNAs in J774A.1 macrophages (at a concentration of 32 μM) [29]. These results allow the authors to suggest that nobiletin could be a candidate for the development of a novel anti-inflammatory or immunomodulatory drug.

Baicalin (**5**), baicalein (**6**) and wogonin (**7**) are flavonoids present in *Scutellaria baicalensis* Georgi (Lamiaceae), a plant used in the treatment of a variety of inflammatory diseases such as bronchitis, nephritis, hepatitis, asthma, and atopic dermatitis [30]. The anti-inflammatory activities of these flavonoids have been attributed to their antioxidant properties and to their ability to inhibit LPS-induced NO production and iNOS gene expression, as well as the increase in TNFα levels by RAW 264.7 cells [31], [32]. Li et al. [33] have demonstrated that baicalin (IC₅₀ values ranging from 15 to 320 μg/mL) and, to a lesser extent, baicalein, significantly inhibited the binding of several chemokines such as CXC, [stromal cell-derived factor (SDF)-1α and IL-8], CC [macrophage inflammatory protein (MIP)-1β, monocyte chemoattractant protein (MCP)-2], and C lymphotactin (Ltn)] to human leukocytes or cells transfected with chemokine receptors. Baicalein also prevented eotaxin production (approximate IC₅₀ value of 1.8 μg/mL) and the mRNA eotaxin expression in human fibroblasts stimulated with IL-4 plus TNFα [34]. Confirming the *in vitro* studies, the co-injection of baicalein with interleukin-8 (IL-8) significantly inhibited IL-8-elicited neutrophil infiltration in rat skin. However, baicalin failed to block CX3C chemokine fractalkine/neurotactin or other cytokines, such as TNFα or IFN-γ [33]. Several studies have demonstrated that most flavonoids, including baicalein (IC₅₀ values ranging from 2.4 to 9.7 μM) inhibit IL-1β-, TNFα- and thrombin-induced endothelial leukocyte adhesion molecule-1 (ELAM-1) and ICAM-1 expression in cultured human umbilical vein endothelial cells (HUVEC) [19], [35]. Sartor et al. [36] have reported that different flavonoids (IC₅₀ values between 0.4 and 450 μM), such as flavones (baicalein), anthocyanidins (delphinidin and pelargonidin), flavanols [epigallocatechin-3-gallate (**8**)], flavonols [morin (**9**) and quercetin] and other compounds with antioxidant properties were highly effective in inhibiting the activities of leukocyte elastase, MMP-2 and MMP-9. Such actions might explain the anti-inflammatory, antiangiogenic, anti-invasive and antimetastatic properties described for these compounds. Krakauer et al. [37] have shown that baicalin (IC₅₀ values ranging from 3 to 50 μg/mL) inhibited the expression and production of IL-1β, IL-6, TNFα, IFNγ, MIP-1α/β in human peripheral blood mononuclear cells under stimulation with superantigenic staphylococcal exotoxins. More recently, Shen et al. [38] reported that baicalein and baicalin were able to decrease fMLP- or PMA-induced accumulation of reactive oxygen intermediates in human neutrophils and monocytes (IC₅₀ values ranging from 1.5 to 64.5 μM). Furthermore, baicalein and baicalin diminished the fMLP-induced increase in surface expression of

Phenolic Compounds I:

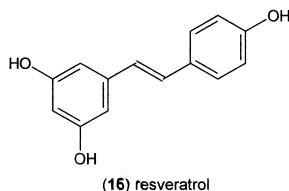
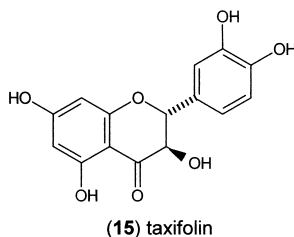
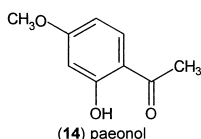
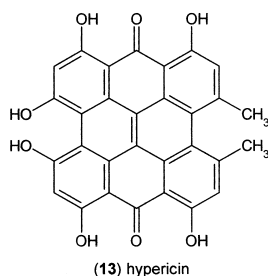
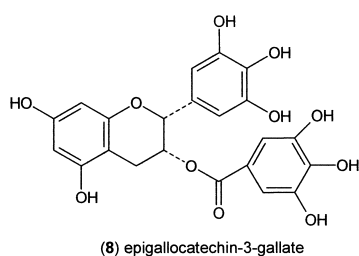


the integrin MAC-1 (CD11b/CD18) and MAC-1-dependent neutrophil adhesion [38]. Baicalein and wogonin (approximate IC₅₀ values of 1 to 40 μM) were also effective in blocking the production and expression of IL-6 and IL-8 in a human retinal pigment epithelial cell line [39].

It has been recently reported that standardised extracts of *Ginkgo biloba* L. (IC₅₀ > 400 μg/mL) (Ginkgoaceae) (EGb 761) and its flavonoid component quercetin (IC₅₀ < 200 μg/mL) inhibited TNFα secretion in LPS-stimulated RAW 264.7 macrophages, by interfering with the phosphorylation and activation of JNK/SAPK and its downstream substrates c-Jun and ATF-2, and ERK1/2 and p38 MAPK. In addition, Wadsworth et al. [40] have shown that *Ginkgo biloba* extract (EGb 761) and quercetin suppress the activation of the transcription factor AP-1. Quercetin (IC₅₀ < 20 μM) [41] and quercetin 3-O-methyl ether from *Rhamnus nakaharai* Hayata (Rhamnaceae) [42], as well as amoradecin (IC₅₀ 28.5 μM) isolated from *Amorpha fruticosa* L. (Fabaceae) [43], markedly inhibited the production of TNFα in LPS-stimulated murine macrophages. Quercetin 3-O-methyl ether also caused a pronounced inhibition of TNFα formation according to an evaluation in LPS/IFN-γ-stimulated murine microglial N9 cells [42]. Luteolin 4'-O-glucopyranoside, cosmosiin, apigenin (**10**), luteolin, quercimeritrin and kaempferol, from *Kummerowia striata* Thunb. (Leguminosae) exhibited concentration-dependent inhibitory effects on IL-5 bioactivity, with mean IC₅₀ values of 3.7, 14.2, 16.4, 18.7, 27.3 and 30.0 μM, respectively [44].

Okabe et al. [45] reported that (-)-epigallocatechin gallate (EGCG), the main constituent of green tea polyphenols, tannins [geraniin (**11**) and corilagin (**12**)] and the aqueous extract of leaves of *Acer nikoense* Maxim. (Aceraceae), a Japanese herbal medicine used for eye and liver diseases, effectively prevented TNFα release in BALB/3T3 cells stimulated with okadaic acid. The mean IC₅₀ values obtained were 26 μM for EGCG, 43 μM for

Phenolic Compounds II:



geraniin, and 76 μM for corilagin. In addition, treatment of animals with geraniin (5 mg/site) and EGCG reduced the percentage of tumour-bearing mice from 80 to 40% and 73 to 0%, and the average number of tumours from 3.8 to 1.1 and 4.2 to 0, following topical application of 7,12-dimethylbenz[a]anthracene (DMBA) plus okadaic acid, respectively [45].

1,7-Bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one and procucumenol present in the rhizomes of the plant *Curcuma zedoaria* L. (Zingiberaceae) were found to be effective in reducing the production of $\text{TNF}\alpha$ in LPS-activated macrophages, with mean IC_{50} values of 12.3 μM and 310.5 μM , respectively [46]. Kang et al. [47] reported that hypericin (13), an active component of *Hypericum perforatum* L. (Hypericaceae), significantly inhibited

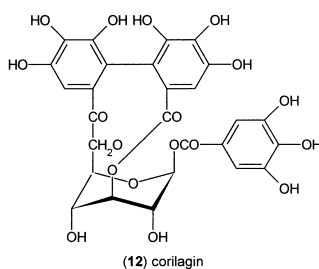
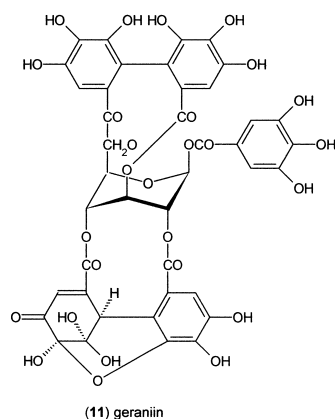
in a concentration-dependent manner IL-12 production in LPS-activated macrophages (IC_{50} 1.45 $\mu\text{g}/\text{mL}$). Hypericin potently inhibited the activation of the IL-12 gene promoter, suggesting that hypericin negatively regulated IL-12 production at the transcription level. Furthermore, Bork et al. [48] have demonstrated that hypericin inhibits PMA- and $\text{TNF}\alpha$ -induced activation of $\text{NF-}\kappa\text{B}$ by a mechanism not involving antioxidant pathways. These results might explain some known biological activities of hypericin, including its reported antirheumatic effects [48].

It has been demonstrated that a methanolic extract of the root cortex of *Paonia suffruticosa* Andrews (Ranunculaceae), known as mudanpi, inhibited in a concentration-related manner the secretion of IL-8 and MCP-1 induced by PMA in human monocytic U937 cells (approximate IC_{50} value of 35 $\mu\text{g}/\text{mL}$) [49]. Chou [50] demonstrated that its main active compound paeonol (2'-hydroxy-4'-methoxyacetophenone) (14), exhibited analgesic and anti-inflammatory effects in the rat model of carrageenan-evoked thermal hyperalgesia. The analgesic and anti-inflammatory effects of paeonol are associated with its ability to inhibit in a concentration-dependent manner the formation of several pro-inflammatory cytokines (such as $\text{TNF}\alpha$, IL-1- β and IL-6), as well as the over-production of NO and PGE_2 . In addition, the effects of paeonol are also associated with an increase in the anti-inflammatory cytokine IL-10, with inhibition of neutrophil infiltration and iNOS and COX-2 protein expression [50]. Thus, paeonol represents a potential candidate for the development of a new anti-inflammatory therapy.

Pycnogenol, a compound isolated from the bark of *Pinus maritime* Mill (Pinaceae) exhibited a marked scavenger activity when evaluated in the murine macrophage cell lines RAW 264.7 and IC-21 stimulated with H_2O_2 and PMA, respectively. Furthermore, pycnogenol was found to be effective in reducing both the production of IL-1 β and the expression of IL-1 β mRNA in LPS-stimulated RAW 264.7 cells, an effect dependent on interference with the transcription factors $\text{NF-}\kappa\text{B}$ and AP-1 [51]. Recently, Bito et al. [52] have shown that taxifolin (15) purified from pycnogenol reduced IFN- γ -induced ICAM-1 protein, as well as mRNA expression in human keratinocytes, by affecting the activation of the transcription factor STAT-1 and the protein tyrosine phosphorylation of Janus kinase (JAK)-1. This suggests that the JAK-STAT pathway might be the molecular site of action of taxifolin [52].

Manthey et al. [53] have shown that the polymethoxylated flavone, 3,5,6,7,8,3',4'-heptamethoxyflavone (IC_{50} 5 μM) inhibits LPS-induced expression and the production of $\text{TNF}\alpha$, the chemokine MIP-1 α and IL-10 in monocytes. The polymethoxylated flavone has been reported to inhibit human phosphodiesterase activity and has been shown to induce a great elevation of cAMP levels in monocytes [53]. Other preliminary studies have reported that different classes of naturally occurring substances, such as stilbenes isolated from *Magnoliae fargesii* Cheng (Magnoliaceae) produced a marked inhibition of the expression of ICAM-1 and VCAM-1 on the surface of THP-1 human monocytes [54], [55], [56]. Stilbenes, such as 3,5-dihydroxy-4'-methoxystilbene and 2,3,4',5-tetrahydroxystilbene 2-O- β -D-glucopyranoside [as well as resveratrol (16)] were also effective in blocking $\text{TNF}\alpha$ -induced cell-cell adhesion between HUVECs and THP-1 cells [55]. Diarylheptanoids, such as 1-(3,5-dimethoxy-4-hydroxyphenyl)-

Phenolic Compounds III:



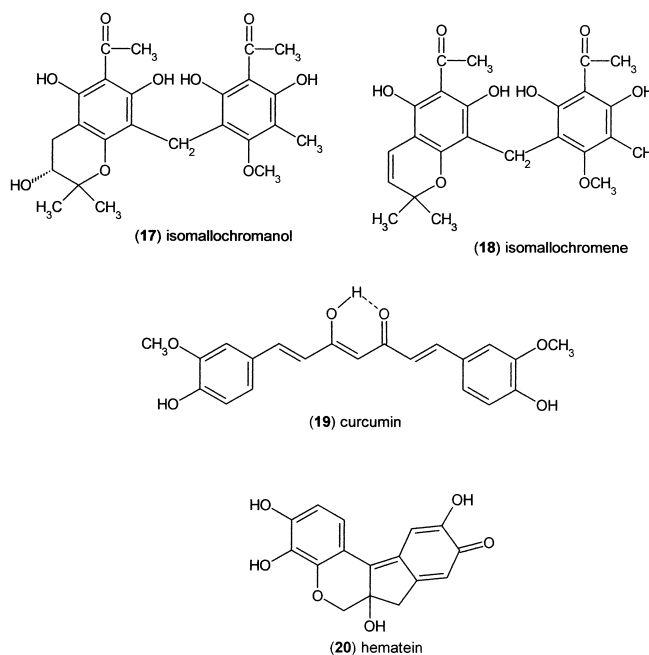
7-phenylhept-1-en-3-one (YPE-01), yakuchinone B and demethylakuchinone B, reduced the adhesion of both the human monocytic cell line U937 and the human eosinophilic cell line EoL-1 to TNF α -treated HUVECs (IC₅₀ values ranging from 29 to 67.2 μ M) [57]. In addition, these compounds were also effective in suppressing both IL-1 β - and TNF α -induced expression of E-selectin, VCAM-1 and ICAM-1 on the surface of the endothelial cells (IC₅₀ values ranging from 17.8 to 40.3 μ M). Since YPE-01 reduces both VCAM-1 and ICAM-1 mRNA induction in TNF α -stimulated endothelial cells, the authors suggest that this compound suppresses adhesion molecule expression at the transcriptional level. Furthermore, YPE-01 given systemically suppressed TPA-induced ear oedema in mice [57].

Piceatannol, another phenolic compound known as a selective inhibitor of the Syk tyrosine kinase significantly prevented TNF α -induced NF- κ B activation in lymphocytes and epithelial cells [58]. Some preliminary studies have indicated that other naturally occurring substances, such as the polyphenol chlorogenic acid, inhibit staphylococcal exotoxin-induced T cell proliferation (by 98%) and the production of IL-1 β , TNF α , IL-6, INF- γ , MCP-1, MIP-1 α and MIP-1 β by human peripheral mononuclear cells [59]. Reynosin exhibited a concentration-dependent inhibition of CINC-1 induction in LPS-stimulated NRK-52E rat kidney epithelioid cells with a mean IC₅₀ value of 1 μ M [60].

Tsuda et al. [61] reported that oral administration of a typical anthocyanin, cyanidin 3-O- β -D-glucoside, suppressed the zymosan-induced inflammatory response in rats. Treatment of cyanidin 3-O- β -D-glucoside also reduced the elevation of NO_x, TNF α , IL-1 β , IL-6 and CINC-1 concentrations. Furthermore, cyanidin 3-O- β -D-glucoside normalised the levels of several acute phase proteins, including α_2 -macroglobulin, albumin and transferrin in the serum of rats treated with zymosan [61]. It has been recently demonstrated that phoroglucinol derivatives, purified from *Mallotus japonicus* Mueller Arg. (Euphorbiaceae) [e.g., isomallotochromanol (**17**) and isomallotochromene (**18**)] are effective in inhibiting the mRNA expression and production of TNF α or IL-6 in RAW 264.7 cells (IC₅₀ values ranging between 0.7 and 30 μ M). These compounds also reduced the formation of TNF α and IL-6 by human blood monocytes activated with LPS, with IC₅₀ values ranging over a similar interval [62]. The effects described for these compounds seem to be dependent on the blockade of NF- κ B activation, but might also include the inhibition of other pro-inflammatory pathways.

Among the polyphenols, the compound obtained from the rhizome of *Curcuma longa* L. (Zingiberaceae), curcumin (**19**), presents high interest as a lead compound to develop new clinically relevant anti-inflammatory drugs. Apart from its effect on inflammatory events (for review, see [63]) this compound significantly blocked IL-12 mediated T cell proliferation and Th₁ differentiation, an action that possibly implies its ability to reduce the production of pro-inflammatory cytokines [64]. Curcumin was found to significantly down-regulate the TNF α -induced increase in MMP-13 mRNA and protein expression in primary human chondrocytes and SW1353 cells by a mechanism involving the inhibition of NF- κ B, c-jun and JNK [65]. Moreover, curcumin significantly inhibited the increase of both IL-1 β and TNF α in a chronic model of inflammation in rats [66]. On account of these

Phenolic Compounds IV:



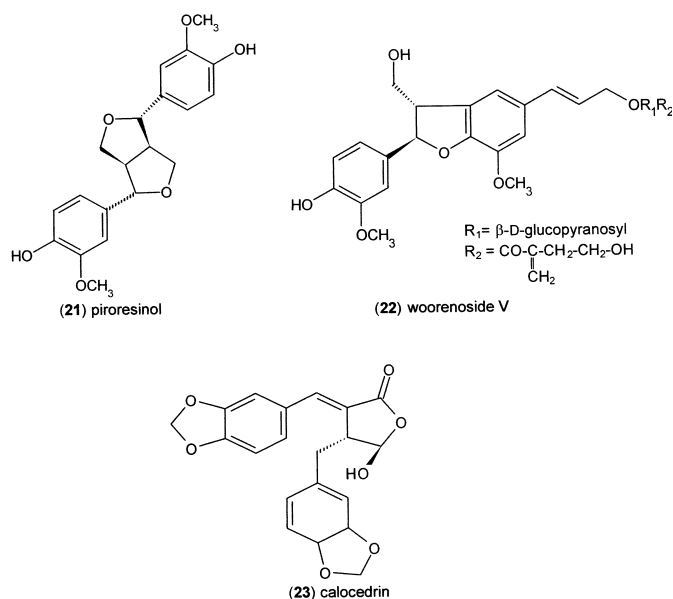
characteristics, curcumin has been submitted to clinical trials. According to a recent evaluation [67], curcumin has been shown to be safe in six human trials designed to test its anti-inflammatory activities. Due to its anti-inflammatory properties, the clinical potential of curcumin for prevention and treatment of cancer was also assessed [68], [69].

Hematein (**20**) is a compound isolated from *Caesalpinia sappan* Linn (Leguminosae), a plant employed in oriental medicine as an analgesic or anti-inflammatory [70]. Oh et al. [71] demonstrated that hematein was efficacious in reducing the expression of VCAM-1 in the aorta of hypercholesterolemic New Zealand rabbits. Hematein also efficiently reduced TNF α -induced VCAM-1 expression in HUVECs [72]. Hematein diminished the increase in VCAM-1 and MCP-1 levels induced by TNF α and oxidised LDL in HUVECs, respectively, as well as reducing TNF α and IL-1 β production by peritoneal macrophages stimulated with LPS plus IFN γ . Furthermore, hematein also reduced the cell surface expression of adhesion molecules, resulting in the inhibition of THP-1 monocyte adhesion to TNF α stimulated HUVECs [73].

Lignans

Cho et al. [74] reported that 10 lignan constituents isolated from the rhizomes of *Coptis japonica* Makino (Ranunculaceae) including pinoresinol (**21**), woorenoside V (**22**) and lariciresinol glycoside had significant inhibitory effects on the TNF α production by LPS-activated RAW 264.7 cells. Five other dihydrobenzofuran neolignans, woorenosides (I–V), obtained from *Coptis japonica* Makino (Ranunculaceae), concentration-dependently blocked TNF α production by LPS-stimulated RAW 264.7 macrophages (IC₅₀ ranging from 15 to 60 μ M) [75]. Other studies have demonstrated that some lignans, including savinin (IC₅₀ = 31.9 μ M) and calocedrin (**23**) (IC₅₀ > 150 μ M) isolated from the heartwood of *Pterocarpus santalinus* Makino (Leguminosae) [76], or pinoresinol

Lignans:



nol, woorenoside V and lariciresinol glycoside (IC_{50} values between 50 and 100 μM) obtained from rhizomes of *Coptis japonica* Makino [76], caused a significant inhibition of $\text{TNF}\alpha$ production in RAW 264.7 macrophages stimulated with LPS. In addition, the same lignans from *Pterocarpus santalinus* Makino (Leguminosae) also inhibited the T-cell proliferation elicited by concanavalin A, without displaying cytotoxic effects [77]. The sesquieolignans, named morinols A and B, isolated from the roots of the Chinese medicinal herb *Morina chinensis* P.Y. Pai (Dipsacaceae) produced a significant inhibition of cytokines formation, including $\text{TNF}\alpha$, IL-4, IL-2 and IFN- γ in human peripheral blood mononuclear cells. However, morinol B ($\text{IC}_{50} > 10 \mu\text{g/mL}$) exhibited a more pronounced activity than its isomer, morinol A [78]. Interestingly, recent data indicate that LPS-treated mice fed a sesamol diet showed a reduction in IL-6 levels in the plasma [79]. Likewise, LPS-treated rats fed with a sesamin-supplemented diet presented reduced $\text{TNF}\alpha$ levels in the plasma [80]. Finally, six lignans isolated from *Magnoliae fargesii* Cheng (Magnoliaceae) produced a marked inhibition of the expression of ICAM-1 and VCAM-1 on the surface of THP-1 human monocytes (IC_{50} values between 20 and 60 μM) [81].

Terpenes

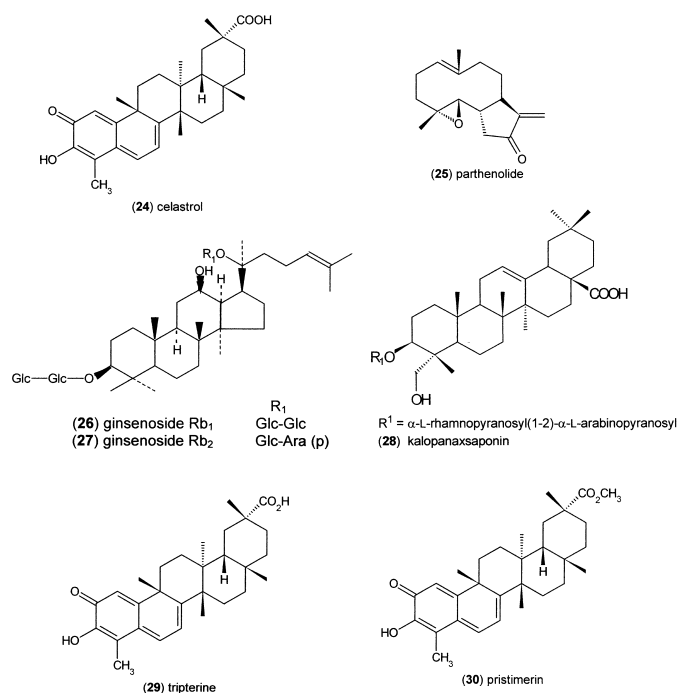
Recently, it has been reported that an extract of *Tripterygium wilfordii* Hook F (Celastraceae) markedly inhibited mRNA synthesis and protein expression of MMP-3 and MMP-13, induced by the pro-inflammatory cytokines IL-1 β , IL-17 and $\text{TNF}\alpha$ as assessed in primary osteoarthritic human or normal bovine chondrocytes ($\text{IC}_{50} > 5 \text{ ng/mL}$) [82]. The authors have suggested that the extract of *T. wilfordii* could be useful as a source and template for novel antiarthritic and cartilage-protective drugs [82]. In addition, celastrol (24) (IC_{50} values between 30 and 100 nM) decreased the production of the pro-inflammatory cytokines $\text{TNF}\alpha$ and IL-1 β in human monocytes and macrophages. Celastrol (3 mg/kg) has also been reported to inhibit rat adjuvant-induced arthritis, thus confirming its reported *in vitro* anti-inflammatory

properties. It has been proposed that celastrol might be useful for improving performance in memory, learning and psychomotor activities, and common features of the neurodegenerative diseases accompanied by inflammation, such as Alzheimer's disease [83]. In addition, it was demonstrated that structurally different sesquiterpene lactones, which possess an α -methylene- γ -lactone function and a conjugated carbonyl group, reduced the production of IL-1 β , $\text{TNF}\alpha$ and IL-6 in adherent mouse peritoneal exudate cells in a concentration-dependent manner (IC_{50} ranging between 0.69 and 1.70 μM) [84]. Schnyder et al. [85] have reported that parthenolide (25) ($\text{IC}_{50} < 10 \mu\text{M}$) blocked the VCAM-1 expression induced by IL-4 in endothelial cells. It was recently shown that parthenolide and the other terpenic compounds isolantolactone and alantolactone decreased the expression of IL-2 in T-lymphocytes [86].

It has been recently reported that ginsenosides Rb1 (26) and Rb2 (27), the major constituents of *Panax ginseng* C.A. Meyer (Araliaceae), are effective in inhibiting $\text{TNF}\alpha$ production in RAW 264.7 and U937 cells stimulated with LPS. The mean IC_{50} values observed were 56.5 and 51.3 μM for Rb1 and 27.5 and 26.8 μM for Rb2, in RAW264.7 and U937 cells, respectively [87]. The inhibitory activity of Rb1 and Rb2 was significantly increased by inhibitors of protein kinase C and A, protein tyrosine kinase or by drugs used for the treatment of arthritis (chloroquine and steroid drugs), but not by AMP phosphodiesterase inhibitors. The authors suggested that ginsenosides could be of clinical interest for the management of $\text{TNF}\alpha$ -mediated diseases, such as arthritis [87].

The terpenic saponin kalopanaxsaponin A (28) (approximate IC_{50} value 5 μM) isolated from *Kalopanax pictus* Nakai (Araliaceae) prevented the formation of $\text{TNF}\alpha$ by RAW 264.7 macrophages stimulated with LPS at concentrations up to 5 μM [88]. Huang et al. [89] reported that tripteryine (29) isolated from *Tripterygium*

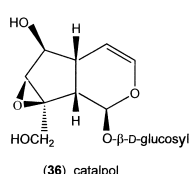
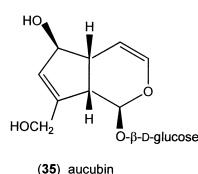
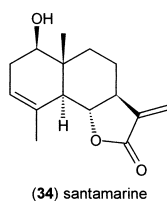
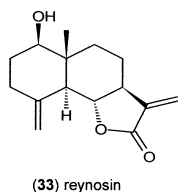
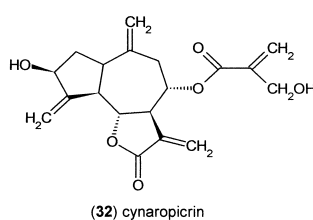
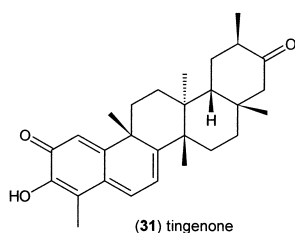
Terpenes I:



wilfordii Hook F. (Celastraceae), or pristimerin (**30**) and tingenone (**31**) isolated from *Maytenus canariensis* Kunk et Sund (Celastraceae) inhibited LPS-stimulated IL-1 β production on human monocytes, with mean IC₅₀ values of 40, 56 and 58 nM. In addition, several sesquiterpene pyridine alkaloids of *T. wilfordii*, named wilforinines A, B and C, as well as several other related compounds, showed significant inhibitory effects on the production of a range of pro-inflammatory cytokines, including TNF α , IL-1 β , IL-4, IL-2 and IFN- γ in human peripheral mononuclear cells (when assessed in the concentration of 10 μ g/mL) [90]. Other sesquiterpene lactones including cynaropicrin (**32**), reynosin (**33**) and santamarine (**34**) from roots of *Saussurea lappa* Radix (Compositae) inhibited TNF α production in LPS-activated RAW 264.7 cells, with IC₅₀ values of 8.2 μ M for cynaropicrin, 87.4 μ M for reynosin and 105 μ M for santamarine [91]. Furthermore, treatment with sulphhydryl (thiol, -SH) compounds such as L-cysteine, dithiothreitol, and 2-mercaptoethanol abrogated the inhibitory effect of cynaropicrin on TNF α production. The authors have proposed that the main inhibitory component of *Saussurea lappa* Radix is cynaropicrin and that its action seems to be mediated through conjugation with SH-groups of target proteins [91].

Jung et al. [92] reported that reynosin (IC₅₀ = 1 μ M) exhibited a concentration-dependent inhibition of CINC-1 formation in NRK-52E rat kidney epithelial cells stimulated with LPS. Moreover, the diterpenes casearinols A and B and casearinones A and B isolated from *Casearia guianensis* J. R. Johnston (Flacourtiaceae) were found to be effective in reducing the expression of ICAM-1 and VCAM-1 in THP-1 human monocytes [93]. Terpinen-4-ol, the main constituent of the essential oil of *Melaleuca alternifolia* (Maiden and Betche) Cheel (Myrtaceae) (tea tree oil) suppressed the formation of TNF α , IL-1 β , IL-8, IL-10 according to an assessment in LPS-activated human peripheral blood monocytes [94].

Terpenes II:



The iridoid glycosides, such as aucubin (**35**), catalpol (**36**), swetiarnin and gardenoside represent a group of cyclopentano[c]pyran monoterpenoids and have been found as constituents of several oriental medicine plants. Aucubin prevented TNF α and IL-6 production in RBL-2H3 stimulated mast cells (IC₅₀ values of 101 and 190 ng/mL, respectively), through a mechanism involving the blockade of NF- κ B activation [95]. Catalposide, the major iridoid glycoside obtained from the stem bark of *Catalpa ovata* G. Don (Bignoniaceae) was effective in preventing the production of TNF α , IL-1 β and IL-6 in LPS-activated macrophages, possibly via NF- κ B inhibition (approximate IC₅₀ value of 50 ng/mL) [96].

Interestingly, the oral administration of cannabinoids has been proposed for the treatment of multiple sclerosis, since cannabinoids seem to stimulate lymphocytes in the CNS by increasing the levels of TNF α [97]. These results are still not conclusive, but open new possibilities for the employment of cannabinoids to treat multiple sclerosis.

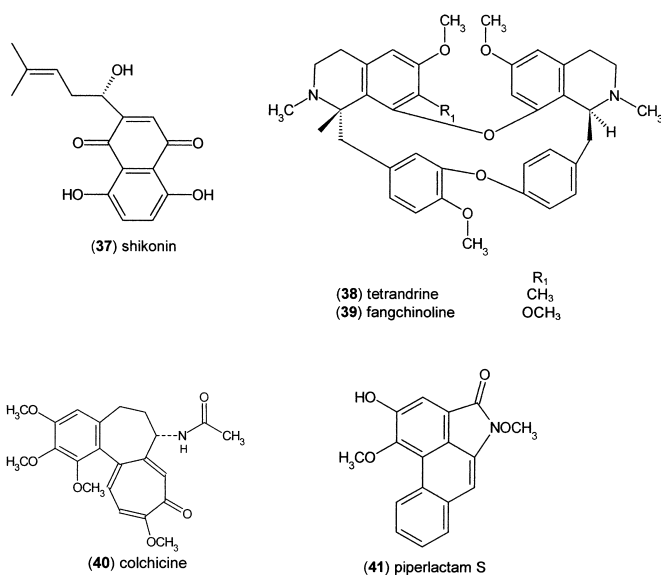
Other Compounds

Physalin B, a seco-steroid isolated from *Physalis angulata* L. (Solanaceae) produced a marked inhibition of the generation of TNF α , IL-6 and IL-12 in macrophages stimulated with LPS and IFN γ (IC₅₀ < 2 μ g/mL). Physalin B (0.5 mg/mouse) also reduced the levels of TNF α in the serum of LPS-treated mice. Nevertheless, physalin B was effective in preventing the septic shock induced by LPS in mice [98].

Shikonin (**37**) was reported to be a component of the traditional Chinese herbal medicine, *Lithospermum erythrorhizon* Sieb. and Zucc. (Boraginaceae), which possesses anti-inflammatory activities [99]. Shikonin blocked the binding of RANTES (regulated upon activation on normal T-cell expressed and secreted) and MIP-1 α , but not SDF-1 α to human monocytes (IC₅₀ values of 3.6 and 2.6 μ M, respectively) [100]. Shikonin also blocked RANTES and MIP-1 α binding to human embryonic kidney (HEK)/293 cells transfected with the stable CC chemokine receptor-1 (CCR1) (IC₅₀ values of 2.63 and 2.57 μ M). Furthermore, shikonin inhibited RANTES-induced CCR1 cell migration, without interfering with CCR1 cell migration induced by epidermal growth factor (EGF). The authors suggest that shikonin appears to be a highly specific antagonist for the CCR1 receptor and holds promise for the future design of more potent, highly selective therapeutics for the treatment of inflammatory autoimmune diseases [100].

Another study has indicated that the specially processed extract of radix *Stephaniae tetrandrae* S. Moore (Menispermaceae) (containing 1.3% of tetrandrine and 0.7% of fangchinoline) and the main alkaloids tetrandrine (**38**) and fangchinoline (**39**) prevented integrin-mediated neutrophil adhesion and transmigration induced by fMLP or leukotriene B₄ (IC₅₀ values between 1 to 5 μ g/mL) [101]. Tetrandrine and fangchinoline also showed a marked inhibitory effect on IL-6 activity *in vitro* (IC₅₀ > 6 μ M) [102]. These two compounds also reduced the increase of IL- β and TNF α levels in human peripheral blood mononuclear cells stimulated with *Staphylococcus aureus* Cowan-1 [103]. Colchicine (**40**) from *Colchicum autumnale* L. (Colchicaceae) presented

Other Compounds:



a significant inhibitory effect on the induction of VCAM-1 in both $\text{TNF}\alpha$ - and $\text{IL-1}\beta$ -stimulated HUVECs [104]. Colchicine was also found to be effective in reducing the levels of IL-6, $\text{TNF}\alpha$ and IL-8 in the serum of patients with familial Mediterranean fever after 2 months of treatment [105]. Likewise, colchicine down-regulated the expression of ICAM-1 and selectin on the surface of HUVECs [106].

The alkaloid piperlactam S (**41**) isolated from *Piper kadsura* (Choisy) Ohwi (Piperaceae) inhibited C5a-induced release of $\text{TNF}\alpha$ and $\text{IL-1}\beta$ in RAW 264.7 macrophages [107]. Finally, very recently Yesilada et al. [108] reported that 55 extracts or fractions obtained from 10 plant species used in Turkish folk medicine for the treatment of rheumatism and related inflammatory diseases, showed inhibitory effects on the production of $\text{IL-1}\alpha/\text{IL-1}\beta$ and $\text{TNF}\alpha$ by human peripheral mononuclear cells stimulated with LPS [108].

Conclusion

Recent data from the literature demonstrate that plenty plant-derived compounds present important anti-inflammatory activities and most of their actions are related to their ability to inhibit cytokine, chemokine or adhesion molecule synthesis and/or action. As discussed before, many inflammatory disorders including sepsis, rheumatoid arthritis and asthma are intimately associated with an imbalance of the cytokine network, as well as to an exaggerated cell influx to the sites of inflammation. In fact, the pharmaceutical industries are currently making tremendous efforts in order to identify new, relevant therapeutic molecules capable of modulating cytokine- and chemokine-activated responses. These agents would be useful not only for the treatment of inflammatory disorders, but also for the control of some other diseases which present an inflammatory origin, such as atherosclerosis and Alzheimer's disease. Although some cytokine modulators, for instance, selective blockers of $\text{IL-1}\beta$ and $\text{TNF}\alpha$ receptors, have been employed clinically, the use of these

drugs presents some disadvantages, especially regarding their high cost, important side effects and route of administration (effective therapy requires at least 2 subcutaneous injections a week). In this context, the development of therapeutic agents based on plant-derived compounds that present anti-cytokine activities would have clear benefits. Thus, plant-derived agents could be used alone or in association with other available anti-inflammatory drugs, allowing a reduction in costs and/or side effects and possibly leading to an increase in effectiveness. However, most of the anti-inflammatory studies on plant-derived compounds have been carried out *in vitro*, and *in vivo* experiments would be required to confirm their efficacy. Furthermore, only a few plant-derived compounds have been submitted to clinical trials to test their potential as anti-inflammatory agents. Finally, as most of the anti-inflammatory compounds are found in several vegetable plant species, it is tempting to suggest that many other plants, not used in folk medicine to treat inflammatory diseases, could present anti-inflammatory properties. Thus, as cytokines are critically involved in most relevant and untreatable inflammatory diseases, large scale studies with plant-derived compounds would constitute a very attractive and relevant field to identify new, effective anti-inflammatory drugs.

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