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Anti-inflammatory activity of extracts from fruits, herbs and spices

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ABSTRACT

Inflammation plays an important role in various diseases with high prevalence within populations such as rheumatoid arthritis, atherosclerosis and asthma. Here we demonstrate the anti-inflammatory activity of various fruits, herbs and spices in a lipopolysaccharide-stimulated macrophage model. These compounds acted by reduction of pro-inflammatory interleukin (IL)-6 or tumour necrosis factor (TNF)-alpha production, enhancement of anti-inflammatory IL-10 production, or reduction of cyclooxygenase-2 or inducible nitric oxide synthase expression. The highest anti-inflammatory potential was detected with chili pepper. Among the plants that improved the secreted cytokine profile were allspice, basil, bay leaves, black pepper, licorice, nutmeg, oregano, sage and thyme. The compounds apigenin, capsaicin, chrysin, diosmetin, kämpferol, luteolin, naringenin, quercetin and resveratrol moderately reduced IL-6 and TNF-alpha secretion. Resveratrol and rosmarinic acid increased secretion of IL-10. Our findings further the idea that a diet rich in fruits, herbs and spices may contribute to the reduction of the inflammatory response and related diseases.

1. Introduction

Inflammation plays an important role in various diseases, such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally. During an inflammatory response, mediators, such as pro-inflammatory cytokines, including interleukin IL-1, tumour necrosis factor (TNF), interferon (INF)-γ, IL-6, IL-12, IL-18 and the granulocyte–macrophage colony-stimulating factor, are released; this response is antagonised by anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, IFN-α and the transforming growth factor. The nuclear factor-κB (NF-κB), transcription factor, also plays an important role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX-2) (Hanada & Yoshimura, 2002; Makarov, 2000); inducible nitric oxide synthase (iNOS) and COX-2 both stimulate the production of large amounts of pro-inflammatory mediators. In chronic inflammation, the negative regulatory mechanism appears to be dysfunctional. Although inflammation is primarily a protective response (against micro-organisms, toxins or allergens, for example), inflammation that is chronic and uncontrolled becomes detrimental to tissues (Gil, 2002).

Since ancient times, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant-derived formulations (Krishnaswamy, 2008; Marc, Nelly, Annick, & Frederic, 2008; Rathore, Mahdi, Paul, Saxena, & Das, 2007; Tapsell et al., 2006). The anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated. Turmeric (*Curcuma longa*), which has traditionally been used for treatment of rheumatic disorders in Indian traditional medicine, exerts both anti-inflammatory and anti-atherosclerotic effects (Krishnaswamy, 2008). Ginger extract (*Zingiber zerumbet*) and its main active compound, 3-*O*-methyl kaempferol, inhibited the production of nitric oxide (NO) and prostaglandin E2 (PGE_2), as well as iNOS expression in a cell culture model. In an *in vivo* model, carrageenan-induced mouse paw oedema was significantly attenuated (Chien, Chen, Lee, Lee, & Wang, 2008). Furthermore, ginger is effective in ameliorating arthritic knee pain (Tapsell et al., 2006). Treatment of LPS-stimulated macrophages with extracts from strawberry (*Fragaria ananassa*), loquat (*Eriobotrya japonica*), mulberry (*Morus alba*) and bitter melon juice (*Momordica charantia*) decreased the secretion of IL-6 and IL-1β pro-inflammatory cytokines and up-regulated the secretion of the anti-inflammatory cytokine IL-10 in a prophylactic cell culture model (Lin & Tang, 2008).

Various plant compounds have also been shown to exhibit anti-inflammatory activity: quercetin inhibits iNOS, COX-2 and C-reactive protein (CRP), and down-regulates NF-κB and TNF-α secretion (Comalada et al., 2006; García-Mediavilla et al., 2007); kaempferol inhibits iNOS, COX-2, CRP and NF-κB (García-Mediavilla et al., 2007; Hämäläinen, Nieminen, Vuorela, Heinonen, & Moilanen, 2007); naringenin down-regulates the secretion of pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNFα and inhibits iNOS expression and NF-κB activation (Bodet, La, Epifano, & Grenier, 2008;

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Hämäläinen et al., 2007); and luteolin inhibits TNFα, IL-6 and IL-1β secretion (Comalada et al., 2006).

In this study, we examined the anti-inflammatory activity of approximately 30 plant extracts and several plant compounds in LPS-stimulated macrophages, a standard model for studying anti-inflammatory drugs or herbs.

2. Materials and methods

2.1. Materials

Apigenin, capsaicin, chrysin, dimethylsulphoxide (DMSO), diosmetin, kämpferol, luteolin, myricetin, naringenin, quercetin, resveratrol, rosmarinic acid, thiazolyl blue tetrazolium bromide (MTT) and LPS from *Escherichia coli* O111:B4 were obtained from Sigma–Aldrich (Darmstadt, Germany). Pomegranate extract (standardised to 40% ellagic acid) was purchased from Styrka Botanics (Hillsborough, US); apple extract (200:1), and holy basil extract (5:1, standardised to tannins) were obtained from Pfannenschmidt (Hamburg, Germany); cinnamon extract (10:1) and thyme extract (7:1) were purchased from Eurochem (Vienna, Austria). Bilberry (standardised to 35% phenols and 10% anthocyanines) was purchased from Pharmalink (Leichlingen, Germany); green coffee and ginseng extract were purchased from Exxentia (Madrid, Spain), and fenugreek extract was obtained from Vivatis Pharma (Hamburg, Germany). Oregano, sage, clove, nutmeg, bay leaves, black pepper, caraway, coriander, cayenne, basil, ginger, marjoram, paprika, allspice, lemon grass, cardamom, anis and rosemary, which were obtained from Kotanyi, were ground but not concentrated extracts (Wolkersdorf, Austria). Rooibos tea, cacao, and licorice were purchased in local Austrian supermarkets or health food shops.

Dulbecco's minimum essential medium (DMEM) was obtained from Biochrom (Berlin, Germany) and foetal calf serum

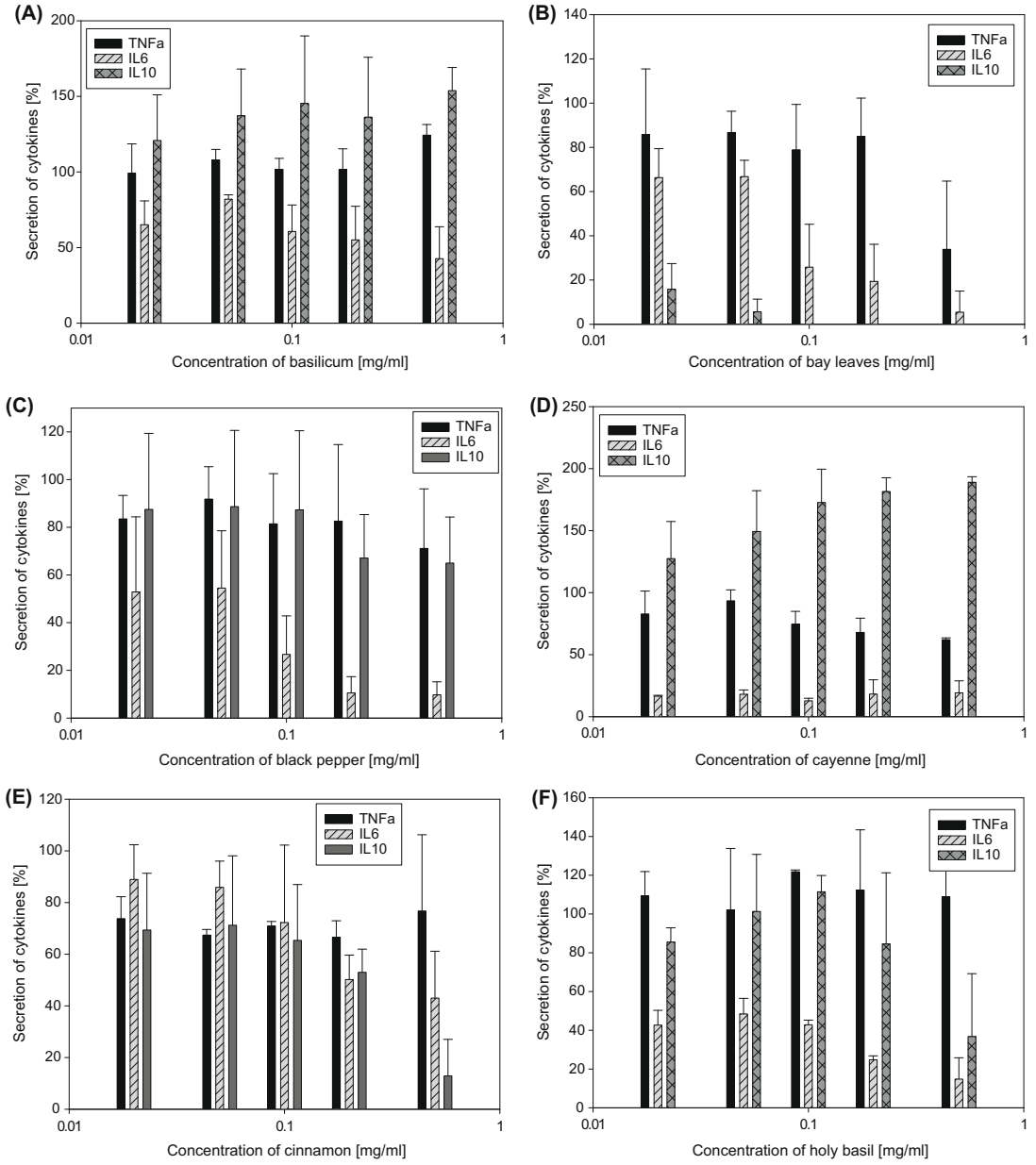

Fig. 1. Influence of various concentrations of plant extracts on the secretion of TNFα, IL-6 and IL-10, as determined by ELISA assays: (A) basil, (B) bay leaves, (C) black pepper, (D) cayenne, (E) cinnamon, (F) holy basil, (G) licorice, (H) nutmeg, (I) oregano, (J) allspice, (K) sage; (L) influence of clove, apple and rooibos tea on IL-6 secretion, and (M) influence of paprika, cardamom, marjoram on IL-10 secretion.

(FCS) was purchased from HyClone (Logan, UT, USA). The DuoSet ELISA Development Systems for IL-6, IL-10 and TNFα cytokines were purchased from R&D Systems (Minneapolis, MN, USA).

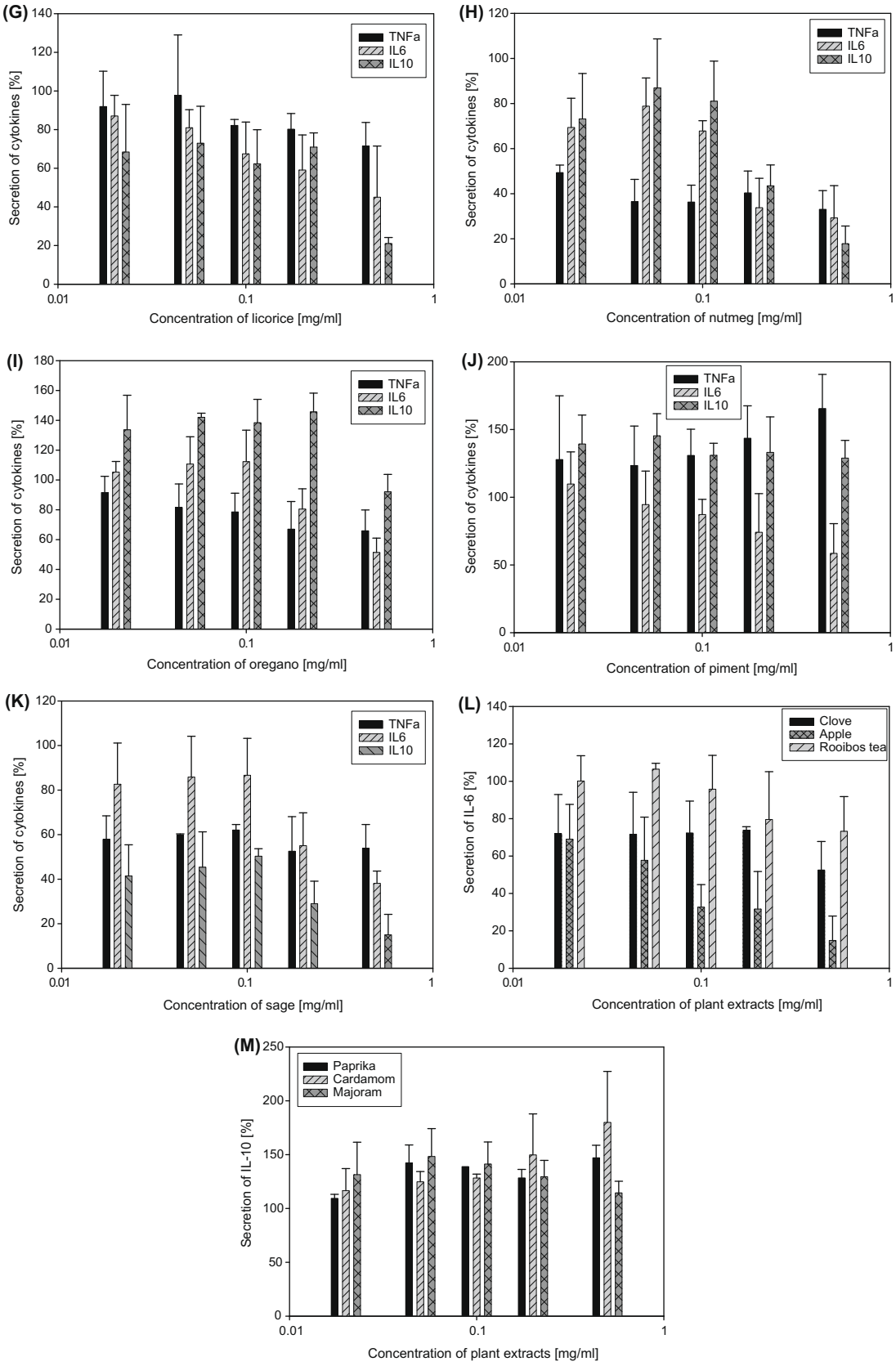

Fig. 1 (*continued*)

2.2. Extraction of the plants/plant powders

Dry powder (100 mg) of plants, herbs or spices was extracted with 1 ml DMSO for 24 h at room temperature, the mixture was clarified by centrifugation for 1 h at 16,000*g*. The clear supernatant was then further diluted with DMSO and used in the inflammation assay.

2.3. Determination of pro- or anti-inflammatory activity

2.3.1. Cell culture

To examine the effect of various plant extracts and compounds on inflammation, we used macrophages stimulated with LPS. RAW 264.7 cells (American Type Culture Collection, ATCC-TIB-71) were seeded at a density of 5×10^5 cells per well in 12 well plates,

Table 1
Characteristics of the plant extracts and the concentration used in the LPS-stimulated macrophage model; the amounts of secreted cytokines, as determined by ELISA and the expression of COX-2 and iNOS by Western blot, were calculated as a percent of the LPS-stimulated positive control cells. The tested material was on a heterogeneous basis. The extracts from local markets and Kontanyi were just ground and not concentrated whereas all extracts from Styrka Botanics, Pfannenschmidt, Eurochem, Pharmalink, Exxentia, and Vivatis Pharma were standardised to a higher content of possibly active ingredients.

Plant extract, ratio of extraction (standardisation)	Species	Plant part	Concentration (mg/ml)	IL-6 (%)	TNF-α (%)	IL-10 (%)	COX-2 (%)	iNOS (%)
Allspice	*Pimenta officinalis*	Fruit	0.5	59 ± 10	166 ± 25	129 ± 13	132 ± 34	24 ± 9
			0.2	74 ± 29	144 ± 24	133 ± 26		
Anise	*Pimpinella anisum*	Fruit	0.5	109 ± 16	120 ± 16	59 ± 24	85 ± 13	92 ± 27
			0.2	110 ± 8	132 ± 10	92 ± 18		
Apple 200:1 (5% quercetin, 30% phloridzin)	*Malus domestica Borkh.*	Fruit	0.5	15 ± 5	102 ± 16	5 ± 1	81 ± 16	101 ± 25
			0.2	32 ± 10	89 ± 17	10 ± 5		
Basil	*Ocimum basilicum*	Leaves	0.5	43 ± 13	124 ± 16	154 ± 15	81 ± 23	67 ± 17
			0.2	55 ± 13	102 ± 27	136 ± 40		
Bay leaves	*Laurus nobilis*	Leaves	0.5	5 ± 2	34 ± 7	0	67 ± 18	112 ± 24
			0.2	19 ± 1	85 ± 17	0		
Bilberry (35% phenols, 15% anthocyanines)	*Vaccinium myrtillus*	Fruit	0.5	99 ± 4	62 ± 17	116 ± 22	111 ± 32	97 ± 27
			0.2	90 ± 14	72 ± 23	118 ± 31		
Black pepper	*Piper nigrum*	Fruit	0.5	10 ± 9	71 ± 25	65 ± 18	86 ± 24	51 ± 16
			0.2	11 ± 7	83 ± 17	67 ± 18		
Cacao	*Theobroma cacao*	Seed	0.5	100 ± 26	123 ± 33	124 ± 27	121 ± 22	48 ± 16
			0.2	114 ± 17	107 ± 22	114 ± 14		
Caraway	*Carum carvi*	Seed	0.5	55 ± 4	62 ± 15	75 ± 22	113 ± 13	107 ± 13
			0.2	67 ± 19	70 ± 6	85 ± 22		
Cardamom	*Elettaria cardamomum*	Seeds	0.5	146 ± 44	125 ± 22	180 ± 47	121 ± 29	84 ± 19
			0.2	110 ± 12	132 ± 36	150 ± 38		
Chili pepper	*Capsicum annuum*	Fruit	0.5	19 ± 8	62 ± 1	189 ± 4	98 ± 24	35 ± 11
			0.2	18 ± 24	68 ± 21	182 ± 11		
Cinnamon 10:1	*Cinnamomum cassia*	Bark	0.5	43 ± 18	77 ± 7	13 ± 14	105 ± 33	56 ± 17
			0.2	50 ± 9	67 ± 6	53 ± 9		
Clove	*Syzygium aromaticum*	Flower bud	0.5	53 ± 15	96 ± 10	110 ± 30	83 ± 26	94 ± 23
			0.2	74 ± 2	95 ± 16	110 ± 31		
Coriander	*Coriandrum sativum*	Seeds	0.5	91 ± 25	91 ± 26	119 ± 21	107 ± 31	71 ± 16
			0.2	98 ± 33	94 ± 22	119 ± 35		
Fenugreek	*Trigonella foenum graecum*	Seeds	0.5	100 ± 16	85 ± 18	90 ± 23	113 ± 25	0
			0.2	94 ± 24	86 ± 1	80 ± 23		
Ginger	*Zingiber officinale*	Rhizomes	0.5	50 ± 14	85 ± 15	68 ± 26	105 ± 22	58 ± 14
			0.2	46 ± 14	93 ± 15	109 ± 31		
Ginseng (15% ginsenosides)	*Panax ginseng*	Root	0.5	88 ± 9	89 ± 14	121 ± 15	102 ± 33	107 ± 27
			0.2	87 ± 6	88 ± 23	126 ± 10		
Green coffee (45% phenolic compounds)	*Coffea arabica*	Seed	0.5	97 ± 34	96 ± 20	121 ± 36	91 ± 14	99 ± 19
			0.2	103 ± 13	93 ± 32	114 ± 20		
Holy basil 5:1	*Ocimum sanctum*	Plant	0.5	15 ± 19	109 ± 27	37 ± 22	123 ± 23	43 ± 16
			0.2	25 ± 2	112 ± 31	85 ± 36		
Lemon grass	*Cymbopogon citratus*	Leaves	0.5	101 ± 10	151 ± 24	111 ± 55	115 ± 22	66 ± 13
			0.2	99 ± 24	105 ± 6	105 ± 7		
Licorice	*Glycyrrhiza glabra*	Root	0.5	45 ± 18	72 ± 12	21 ± 14	99 ± 21	0
			0.2	59 ± 18	80 ± 8	71 ± 33		
Marjoram	*Origanum majorana*	Leaves	0.5	80 ± 13	101 ± 9	114 ± 11	107 ± 19	34 ± 7
			0.2	83 ± 25	107 ± 22	130 ± 15		
Nutmeg	*Myristica fragrans*	Fruit	0.5	30 ± 12	33 ± 21	18 ± 13	87 ± 20	106 ± 18
			0.2	34 ± 13	40 ± 16	44 ± 9		
Oregano	*Origanum onites*	Leaves	0.5	51 ± 6	66 ± 14	92 ± 12	102 ± 21	0
			0.2	81 ± 10	67 ± 18	146 ± 13		
Paprika	*Capsicum annuum*	Fruit	0.5	71 ± 6	98 ± 21	147 ± 12	117 ± 23	21 ± 5
			0.2	70 ± 4	100 ± 23	128 ± 8		
Pomegranate (40% ellagic acid)	*Punica granatum*	Fruit	0.5	101 ± 28	338 ± 77	129 ± 28	128 ± 27	119 ± 33
			0.2	117 ± 7	253 ± 92	121 ± 36		
Rooibos tea	*Aspalathus linearis*	Leaves	0.5	73 ± 30	112 ± 9	67 ± 17	134 ± 18	93 ± 19
			0.2	80 ± 23	113 ± 24	79 ± 27		
Rosemary	*Rosmarinus officinalis*	Plant	0.5	92 ± 6	110 ± 24	0 ± 11	113 ± 13	25 ± 7
			0.2	82 ± 17	101 ± 23	7 ± 14		
Sage	*Salvia officinalis*	Leaves	0.5	38 ± 5	54 ± 12	15 ± 5	107 ± 27	0
			0.2	55 ± 15	53 ± 16	29 ± 12		
Thyme 7:1 (5% rosmarinic acid)	*Thymus vulgaris*	Plant	0.5	64 ± 12	77 ± 23	13 ± 2	87 ± 24	100 ± 23
			0.2	99 ± 33	78 ± 37	49 ± 11		

and incubated for 24 h at 37 °C. On the following day, test substances in <0.1% DMSO solution in DMEM were added, and cells were incubated for a further 3 h at 37 °C before LPS was added at a final concentration of 1 μg/ml. Cells were pretreated before addition of LPS because this way the best results were achieved in prestudies. The cells were then incubated for a further 24 h at 37 °C. On the third day, the media was removed and centrifuged at 1500*g* to remove cells; the supernatant was aliquoted and stored at −20 °C prior to analysis by ELISA. For Western blot analysis, cultured cells were harvested, washed, and lysed, and extracts were stored at −20 °C prior to analysis. Cells which were not treated with LPS served as a negative control and cells incubated with DMSO and LPS served as a positive control.

2.3.2. Elisa

The concentrations of TNF-α, IL-6, and IL-10, in 100 μl of cell supernatant each, were determined by ELISA assay according to the manufacturer's protocol (R&D Systems). All incubation steps were performed at room temperature. The optical density at 450 nm, corrected by the reference wavelength 570 nm, was measured with a Genios Pro microplate reader (Tecan, Crailsheim, Germany).

2.3.3. Western blot

The protein extracts were separated by sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked by incubation in phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA) for 2 h at room temperature or overnight at 4 °C. After one wash with PBS containing 0.1% Tween 20, the membrane was incubated with primary antibody (mouse monoclonal anti-NFκb, goat polyclonal anti-COX-2 or rabbit polyclonal anti-iNOS antibody) for 2 h at room temperature, followed by a 1-h incubation with the appropriate secondary antibody (horseradish

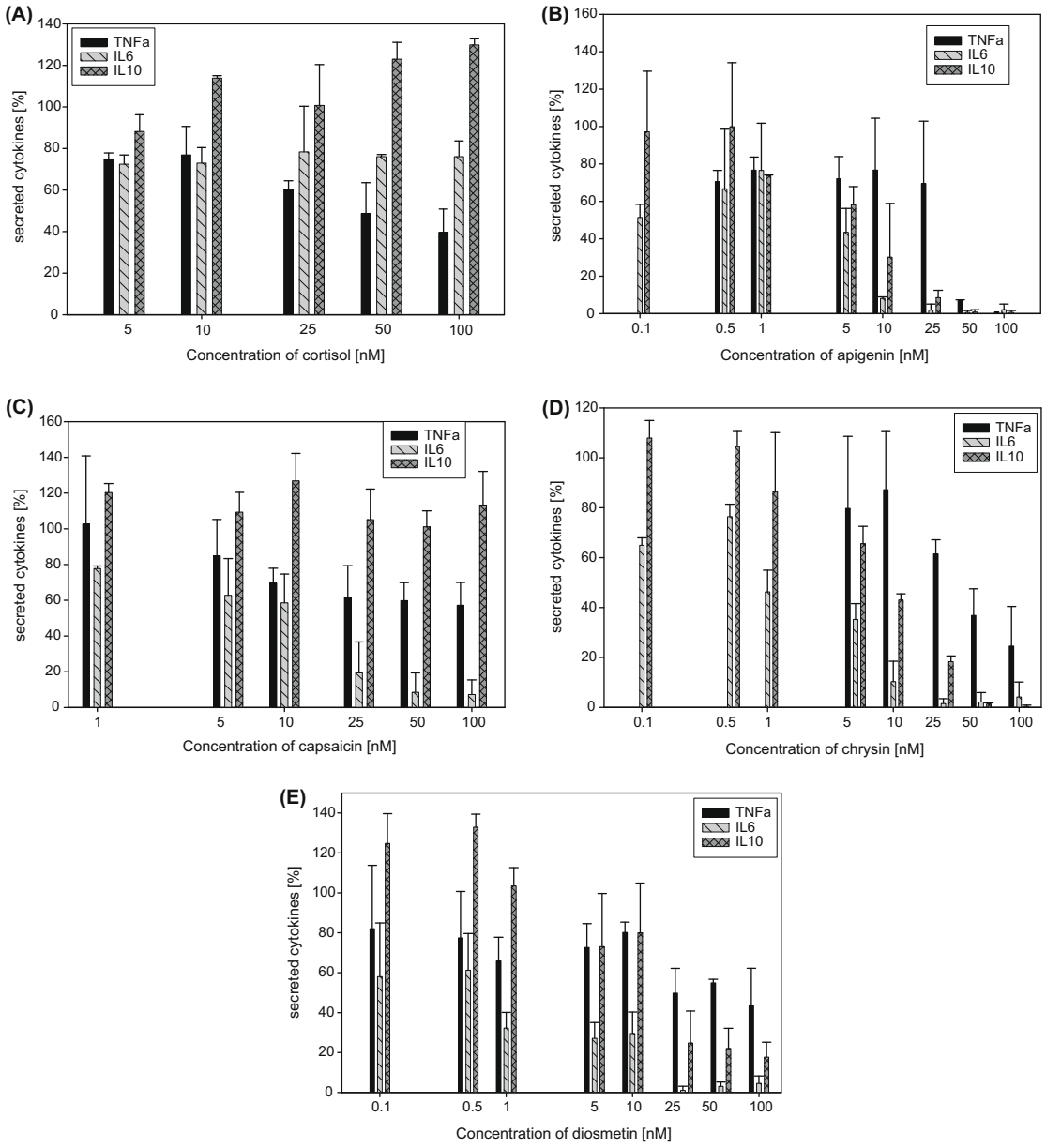

Fig. 2. Influence of various plant compounds on the secretion of TNFα, IL-6 and IL-10, as determined by ELISA assays: (A) cortisol, (B) apigenin, (C) capsaicin, (D) chrysin, (E) diosmetin, (F) kämpferol, (G) luteolin, (H) quercetin, (I) resveratrol, and (J) rosmarinic acid.

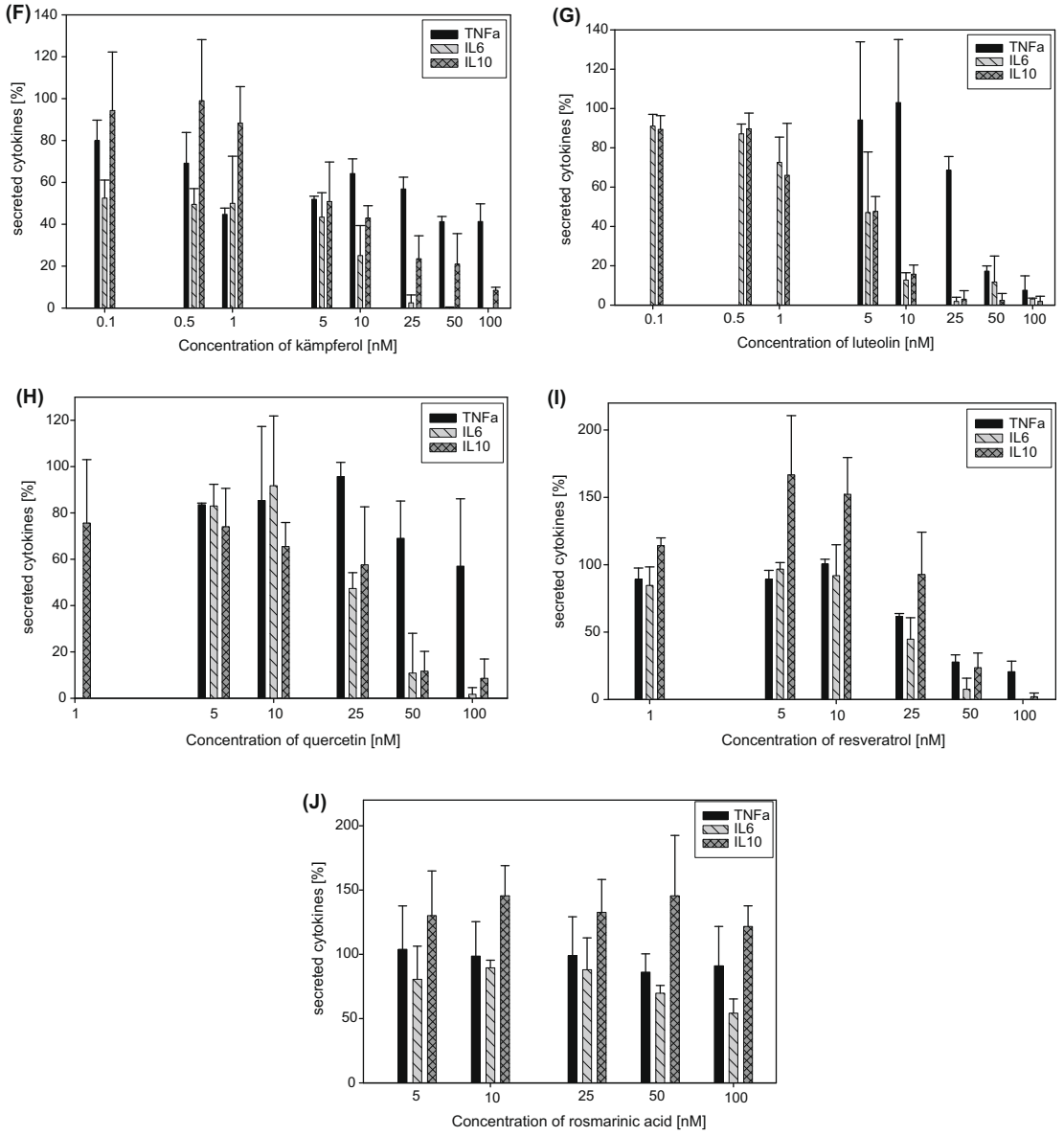

Fig. 2 (*continued*)

peroxidase (HRP)-conjugated anti-goat, anti-rabbit or anti-mouse antibody) at room temperature. The blots were developed using the Lumi Phos reagent and quantified with a Lumi Imager (Boehringer Mannheim, Mannheim, Germany).

2.3.4. MTT assay

Simultaneous with the ELISA assay, the viability of LPS-stimulated cells was assessed by a MTT assay, based on the mitochondrial-dependent reduction of MTT to formazan. After removing the supernatant for ELISA analysis, MTT was added to the cells, and the cells were incubated for 3 h at 37 °C. The supernatant was then removed, and the cells were lysed with lysis buffer (10% SDS in 0.01 N HCl). The optical density at 570 nm, corrected by the reference wavelength 690 nm, was measured using a Genios Pro microplate reader.

2.4. Calculation of the anti-inflammatory activity

Standard curves, with defined cytokine concentrations, were calculated within each ELISA assay in order to determine the concentration of the secreted cytokines. The calculated concentrations of cytokines were normalised to MTT values to reduce any variation from differences in cell density. For a positive control, cells were treated with only LPS and the resulting amount of secreted cytokines was defined as 100%. The results from the experimental compounds were then calculated as a percent of this value. The entire inflammation assay, starting with cell seeding and LPS-induction, was performed (triplicate or quadruplicate) on individual days. ELISA and MTT analyses were performed in duplicate within one assay. Intervariation was in the same range as intravariation.

We also determined the IC_{50}, which is the concentration of the test compounds that results in a half-maximal reduction of cytokine secretion. The values from the normalised cytokine secretions were plotted against the concentrations of the test compounds or plant extracts. Curve fitting was performed using a logistic dose–response model (Eq. (1)) of the Table Curve 2D software (Jandel Scientific, Erkrath, Germany). This non-linear equation uses a Levenburg–Marquard algorithm (Jungbauer & Graumann, 2001):

$$y = a + \frac{b}{1 + (c/x)^d}, \quad (1)$$

where a represents the baseline, b is the difference between the plateau of the curve and the baseline, c is the transition centre of the curve, which is the concentration that causes 50% reduction of cytokine secretion, and d is the transition zone, and is a measure of positive or negative cooperativity.

3. Results

3.1. Cytokine levels in response to treatment with plant extracts and compounds

In this study, we used LPS-stimulated macrophages as a model for testing plant extracts and several compounds for pro- or anti-inflammatory activity. Macrophages were treated with various compounds and LPS, and the concentrations of secreted IL-10, IL-6 and TNF-α in the supernatant were determined. The plant extracts showed a considerable range of influence on cytokine secretion. The plant extracts with the most significant anti-inflammatory activity are shown in Fig. 1A–M, and the results from all tested plant extracts are summarised in Table 1. Secretion of the pro-inflammatory cytokine, IL-6, was significantly reduced (by at least 25%) upon incubation with allspice, apple, basil, bay leaves, black pepper, caraway, chili pepper, cinnamon, clove, ginger, holy basil, licorice, nutmeg, paprika and sage extracts at both 0.2 and 0.5 mg/ml concentrations, and by 0.5 mg/ml of oregano, rooibos tea and thyme extract. Secretion of the pro-inflammatory cytokine TNF-α, was decreased by at least 25% upon incubation with 0.2 or 0.5 mg/ml of bilberry, caraway, chili pepper, licorice, nutmeg, oregano, sage and thyme extract. TNF-α levels also decreased upon treatment with 0.5 mg/ml of bay leaf, cinnamon and black pepper extract. Pomegranate extract increased the TNF-α secretion to 250–340%. Allspice, basil, cardamom, chili pepper, ginseng, marjoram, oregano, paprika and pomegranate enhanced the secretion of the anti-inflammatory cytokine IL-10. Interestingly, oregano extract only enhanced the secretion of IL-10 when using 0.2 mg/ml but not when using 0.5 mg/ml. Various plant extracts reduced IL-10 secretion, namely anise, apple, bay leaves, black pepper, cinnamon, ginger, holy basil, licorice, nutmeg, rooibos tea, rosemary, sage and thyme. An IC_{50} value could only be determined for the reduction of IL-6 production by nutmeg (IC_{50} = 135 ± 23 μg/ml), black pepper (IC_{50} = 97 ± 38 μg/ml), apple (IC_{50} = 82 ± 29 μg/ml), bay leaves (IC_{50} = 86 ± 36 μg/ml) and holy basil (IC_{50} = 172 ± 31 μg/ml).

As a positive control, cells were incubated with cortisol, an anti-inflammatory hormone. A significant reduction of TNF-α secretion, a significant increase of IL-10 production and a slight reduction of IL-6 production were observed when incubating cells with 50 or 100 nM cortisol (Fig. 2A).

The compounds apigenin, capsaicin, chrysin, diosmetin, kämpferol, luteolin, myricetin, naringenin, quercetin, resveratrol and rosmarinic acid (Figs. 2B–J and 3A–K) reduced IL-6 secretion at concentrations of 50 and 100 nM. The most efficient reduction of IL-6 production was in response to chrysin (IC_{50} = 4 ± 2 nM), followed by diosmetin (IC_{50} = 4 ± 6 nM), apigenin (IC_{50} = 5 ± 0.4 nM), luteolin (IC_{50} = 5 ± 0.7 nM), kämpferol (IC_{50} = 9 ± 0.4 nM), capsaicin (IC_{50} = 15 ± 3 nM), resveratrol (IC_{50} = 25 ± 2 nM) and quercetin (IC_{50} = 26 ± 3 nM). Apigenin, capsaicin, chrysin, diosmetin, kämpferol, luteolin, naringenin, quercetin and resveratrol further re-

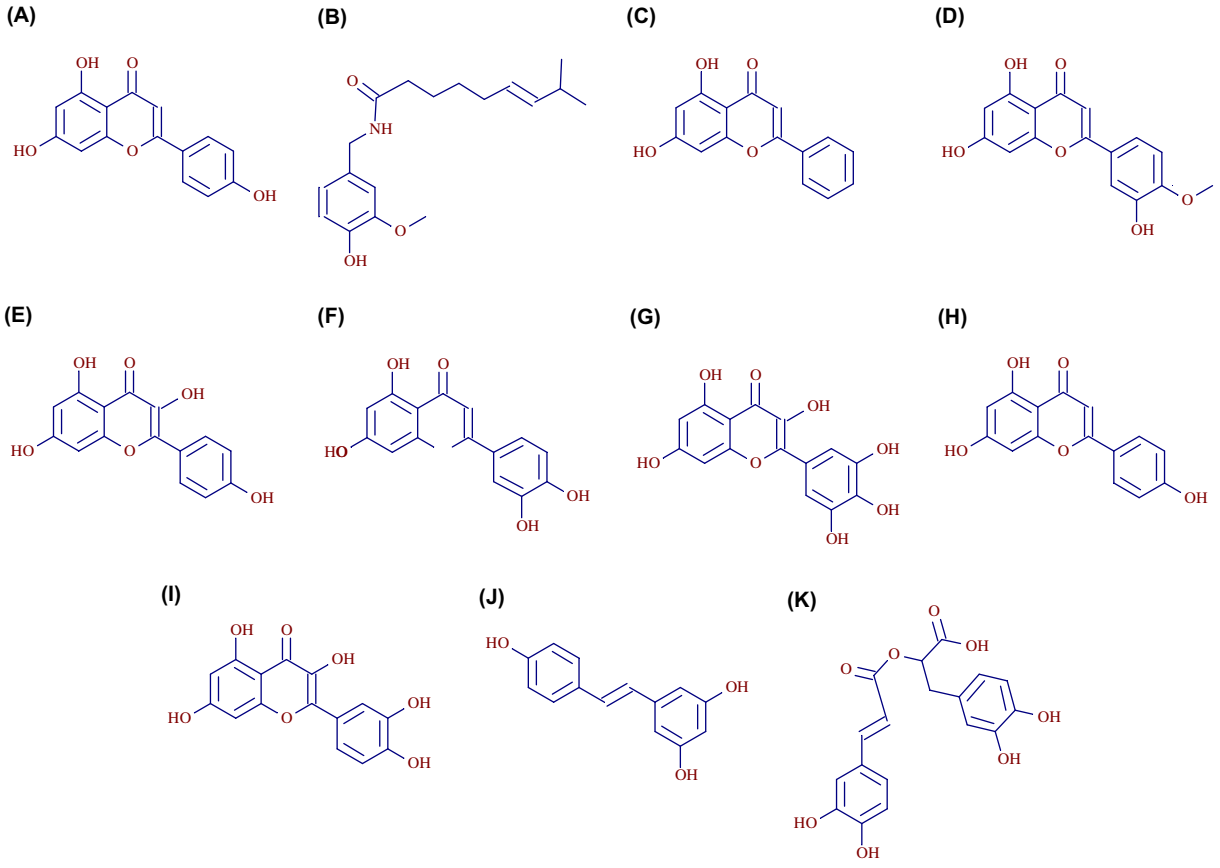

Fig. 3. Structures of (A) apigenin, (B) capsaicin, (C) chrysin, (D) diosmetin, (E) kämpferol, (F) luteolin, (G) myricetin, (H) naringenin, (I) quercetin, (J) resveratrol, and (K) rosmarinic acid.

duced TNF-α secretion (Fig. 2B–G). The IC_{50} for TNF-α reduction could only be determined for chrysin, resveratrol and luteolin, as the other substances did not reach a minimum plateau. The IC_{50} values of these three substances fell within a close range: 26 ± 3 nM for chrysin, 26 ± 3 nM for resveratrol, and 28 ± 5 nM for luteolin. High concentrations of resveratrol were found to decrease IL-10 secretion, while low levels increased IL-10 secretion (Fig. 2H). Rosmarinic acid significantly increased the secretion of IL-10 (Fig. 2I).

3.2. COX-2 and iNOS expression in response to treatment with plant extracts and compounds

In parallel with the ELISA assays, we performed Western blot analysis, to assess the influence of various plant extracts or substances on the expression of COX-2 and iNOS. Plant extracts were used at a concentration of 0.5 mg/ml, and the compounds at a concentration of 100 nM. Only bay leaves significantly reduced the expression of COX-2 by more than 25%, whereas nutmeg, black pepper, basil, thyme, apple, clove and anis slightly reduced COX-2 expression (Fig. 4A, Table 1). Chili pepper, coriander, licorice, sage, oregano, black pepper, basil, rosemary, ginger, marjoram, paprika, cacao, allspice, lemon grass, cinnamon, fenugreek, holy basil, and cortisol all decreased the expression of iNOS (Fig. 4B). Notably, licorice, sage, fenugreek and oregano completely inhibited iNOS expression.

The extracts of allspice, pomegranate and rooibos tea increased the expression of COX-2 by more than 25%.

The anti-inflammatory hormone, cortisol, reduced the expression of iNOS, but not COX-2 levels. Apigenin and luteolin inhibited the expression of COX-2, and apigenin, chrysin, diosmetin, kämpferol, luteolin, quercetin and reveratrol inhibited iNOS expression (Fig. 4C and D, Table 2).

4. Discussion

Studies have demonstrated an association between the typical Western diet rich in refined starches, sugar, saturated and transf-

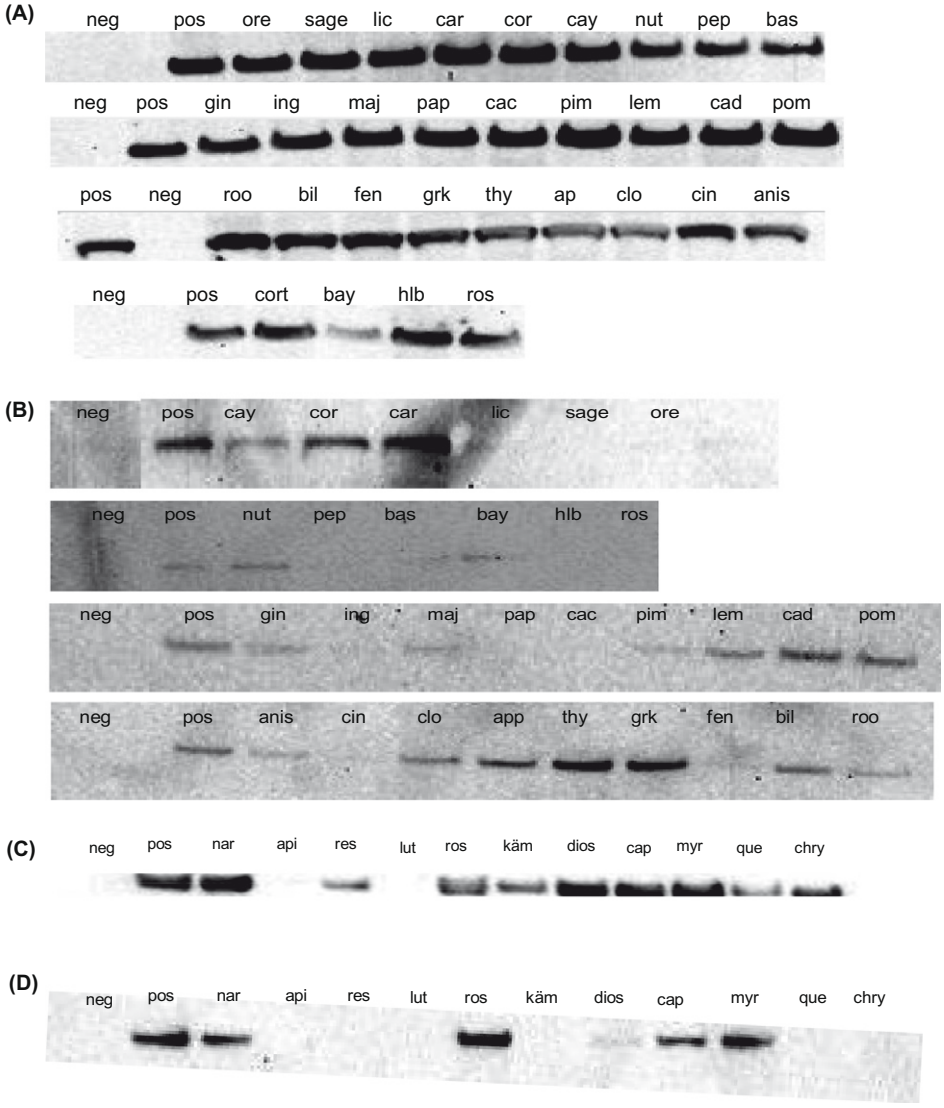

Fig. 4. Expression of COX-2 and iNOS in response to various plant extracts. Western blot analysis of (A) COX-2 and (B) iNOS in macrophages incubated with DMSO only (neg), LPS only (pos) or LPS and allspice (pim), anis, apple (ap), black pepper (pep), basil (bas), bay leaves (bay), bilberry (bil), cacao (cac), caraway (car), cardamom (cad), cay (chili pepper), cinnamon (cin), clove (clo), coriander (cor), cortisol (cort), fenugreek (fen), ginseng (gin), green coffee (grk), holy basil (hlb), ginger (gig), lemongrass (lem), licorice (lic), majoram (maj), nutmeg (nut), oregano (ore), paprika (pap), pomegranate (pom), rooibos tea (roo), rosemary (ros), sage (sage), or thyme (thy). Western blot analysis of (C) COX-2 and (D) iNOS in macrophages incubated with DMSO only (neg), LPS only (pos) or LPS and naringenin (nar), apigenin (api), resveratrol (res), luteolin (lut), rosmarinic acid (ros), kämpferol (käm), diosmetin (dios), capsaicin (cap), myricetin (myr), quercetin (que) or chrysin (chry).

Table 2
Substances from plant extracts were tested in the LPS-stimulated macrophage model at various concentrations (100 or 50 nM); the amounts of secreted cytokines, as determined by ELISA, and the expression of COX-2 and iNOS, by Western blot, were calculated as a percent of the LPS-stimulated positive control cells.

Substance	CAS no.	Concentration (nM)	IL-6 (%)	TNF-α (%)	IL-10 (%)	COX-2 (%)	iNOS (%)	Occurrence (according to literature)
Apigenin	520-36-5	100	2 ± 3	1 ± 0	1 ± 6	0	0	Sage, marjoram, thyme (Proestos, Chorianopoulos, Nychas, & Komaitis, 2005)
		50	1 ± 1	7 ± 0	1 ± 0			
Capsaicin	404-86-4	100	7 ± 8	57 ± 12	113 ± 18	107 ± 19	121 ± 18	Chili pepper
		50	9 ± 10	60 ± 10	101 ± 12			
Chrysin	480-40-0	100	4 ± 6	35 ± 19	0	108 ± 22	0	Passion fruit (Beaumont et al., 2008)
		50	2 ± 4	37 ± 11	1 ± 0			
Cortisol	53-06-5	100	76 ± 7	40 ± 11	130 ± 3	105 ± 27	65 ± 17	
		50	76 ± 1	54 ± 22	123 ± 8			
Diosmetin	520-34-3	100	5 ± 9	43 ± 19	17 ± 15	112 ± 18	0	Oregano (Hawas, El-Desoky, Kawashty, & Sharaf, 2008; Mueller et al., 2008), rosmary (Peng, Yuan, Liu, & Ye, 2005)
		50	3 ± 2	55 ± 2	22 ± 15			
Kämpferol	520-18-3	100	0	41 ± 8	8 ± 3	103 ± 22	0	Pomegranate fruit, dill, chive (Justesen & Knuthsen, 2001)
		50	0	41 ± 2	21 ± 15			
Luteolin	491-70-3	100	3 ± 18	7 ± 15	2 ± 0	0	0	Marjoram, sage, rosemary (Proestos et al., 2005), thyme, parsley (Justesen & Knuthsen, 2001)
		50	11 ± 15	17 ± 19	2 ± 0			
Myricetin	529-44-2	100	43 ± 19	102 ± 28	80 ± 12	105 ± 17	121 ± 23	Bilberry (Ehala, Vaher, & Kaljurand, 2005)
		50	74 ± 23	101 ± 12	83 ± 18			
Naringenin	67604-48-2	100	17 ± 14	73 ± 15	110 ± 13	111 ± 19	96 ± 21	Tomatoes, grapefruit, orange, strawberry (Harnly et al., 2006)
		50	65 ± 7	72 ± 7	103 ± 4			
Quercetin	849061-97-8	100	2 ± 3	57 ± 2	9 ± 8	92 ± 17	0	Bilberry (Ehala et al., 2005), dill, bay leaf, parsley, chive (Justesen & Knuthsen, 2001), apple
		50	11 ± 1	69 ± 16	12 ± 8			
Resveratrol	501-36-0	100	0	21 ± 12	2 ± 2	91 ± 24	0	Bilberry (Ehala et al., 2005)
		50	8 ± 8	28 ± 14	24 ± 7			
Rosmarinic acid	20283-92-5	100	54 ± 11	91 ± 31	122 ± 24	102 ± 13	102 ± 26	Thyme (Proestos et al., 2005), marjoram, sage, rosemary (Wang, Provan, & Helliwell, 2004)
		50	70 ± 6	86 ± 14	145 ± 23			

atty acids and poor in fruits, vegetables, fibre, $\omega-3$ fatty acids and whole grains and an increased tendency towards inflammatory disorders and related diseases, such as cardiovascular diseases, arthritis or diabetes (Giugliano, Ceriello, & Esposito, 2006). In order to reduce inflammation, the major focus has been on maintaining a low ratio of $\omega-6$ to $\omega-3$ fatty acids. In addition, a diet rich in fruits and vegetables has been negatively correlated with various diseases that are associated with inflammatory disorders (Giugliano et al., 2006; Krishnaswamy, 2008; Lin & Tang, 2008). Several dietary polyphenols were shown to ameliorate inflammatory stages via inhibition of COX-2, NF-κB, NOS or peroxisome proliferator-activated receptor (PPAR) activation (Yoon & Baek, 2005). In this study, we screened plant extracts and compounds for anti-inflammatory activity, using LPS-stimulated macrophages, a standard model for studying anti-inflammatory drugs or herbs. The results from our study provide further evidence for the anti-inflammatory activity of several herbs and spices and their compounds.

Several plant extracts and compounds exhibited anti-inflammatory activity by elevating anti-inflammatory IL-10 production, reducing pro-inflammatory IL-6 or TNF-α production, or reducing the expression of iNOS and COX-2. The highest anti-inflammatory effect was detected with chili pepper, which moderately enhanced the IL-10 secretion to 190%, slightly reduced TNF-α secretion to 62% and iNOS expression to 35%, and highly reduced IL-6 secretion to less than 20%. The chili pepper compound capsaicin also positively influenced the profile of the secreted cytokines and reduced iNOS expression. Our results are consistent with those in the literature, which show that capsaicin modulated NF-κB- and IL-8 pathways, and thus inhibited IL-8 production of *Helicobacter pylori*-induced gastric epithelial cells (Lee et al., 2007). Capsaicin further inhibited the production of TNF-α, which might be mediated by PPARγ activation (Park et al., 2004).

We found that black pepper strongly reduced IL-6 production and the expression of iNOS and COX-2. According to the literature, piperine also functioned as an anti-inflammatory compound *in vivo* by suppression of IL-6 production and PGE_2, which was shown in an arthritis model (Bang et al., 2009).

Licorice extract showed slightly anti-inflammatory activity via reduction of IL-6 and TNF-α but also IL-10. In another study using licorice extract, in addition to the inhibition of IL-6 and TNF-α, IL-1β and IL-8 were reduced and NF-κB p65 phosphorylation was inhibited, suggesting its potential for treatment of periodontitis (Bodet, La, Gafner, Bergeron, & Grenier, 2008). In mice, asthmatic features were ameliorated by treatment with licorice extract via inhibition of ovalbumin-induced immediate airway constriction, lung inflammation, and infiltration of eosinophils in the peribronchial and perivascular areas, and decrease in IL-4, IL-5 and eosinophil levels (Ram et al., 2006).

Pomegranate extract increases the TNF-α secretion and the IL-10 secretion, simultaneously, and thus it is not clear if it increases or reduces inflammation.

Nutmeg extract was the most potent inhibitor of TNF-α production, and it also potently inhibited IL-6 and IL-10 production and COX-2 expression. According to the literature, macelignan was demonstrated to be an active anti-inflammatory compound in hippocampal neuronal and primary microglial cells (Ma et al., 2009).

In this study, sage improved the anti-inflammatory profile of the secreted cytokines and inhibited the expression of iNOS. According to the literature, carnosic acid and carnosol, the anti-inflammatory compounds, inhibited the formation of pro-inflammatory leukotrienes and 5-lipoxygenase (Poeckel et al., 2008).

Interestingly, the most active extracts, chilli pepper, black pepper, licorice, nutmeg and sage, were just extracts from ground raw material and not concentrated extracts. This means that the mentioned spices are either very active in comparison with the other extracts or that, in the standardised extracts, the putative anti-inflammatory compounds were not enriched.

We found that the anti-inflammatory hormone cortisol elevated IL-10 secretion, reduced IL-6 and TNF-α production, and inhibited iNOS expression. Notably, several compounds were more efficient in the reduction of IL-6 and TNF-α secretion compared to cortisol (apigenin, capsaicin, chrysin, diosmetin, kämpferol, luteolin, myricetin, naringenin and quercetin); these compounds, however, did not simultaneously increase IL-10 secretion. Cortisol and other corticosteroids function mainly via suppressing multiple inflammatory genes that are involved in chronic inflammation (Barnes, 2006).

In conventional therapy, steroidal and non-steroidal anti-inflammatory drugs that inhibit COX are used to treat acute inflammation, but are unsuccessful at curing chronic inflammatory diseases, such as rheumatoid arthritis or osteoarthritis. Furthermore, these compounds exhibit several undesired side effects. For example, corticosteroids are effective for treating asthma, but fail to treat chronic obstructive pulmonary disease and severe asthma (Barnes, 2006). Therefore, alternative treatments, with safer compounds, are needed (Yoon & Baek, 2005). Based on the positive results from effects on cytokine profiles and inhibition of iNOS and COX-2 expression, several of plant extracts tested in this study could potentially be used as food supplements with the purpose of providing anti-inflammatory effects.

Inflammation not only plays a role in the inflammatory diseases mentioned above, but also in the progression of cancer. Several inflammatory stages have been shown to predispose patients to cancer, such as inflammatory bowel disease, predisposing patients to colorectal cancer, *H. pylori*-induced gastritis to gastric cancer, or prostatitis to prostate cancer (Balkwill, Charles, & Mantovani, 2005).

Furthermore, a diet rich in antioxidants and anti-inflammatory compounds derived from fruits and vegetables may lower the risk of developing age-related neurodegenerative diseases, such as Alzheimer's or Parkinson's disease (Joseph, Shukitt-Hale, & Lau, 2007).

The results from our study demonstrate improved anti-inflammatory response in a LPS-stimulated macrophage model upon treatment with chili pepper, allspice, basil, bay leaves, black pepper, licorice, nutmeg, oregano, sage or thyme via reduction of IL-6 or TNF-α production, enhancement of IL-10 production, or reduction of expression of COX-2 or iNOS. These findings further the idea that a diet rich in fruits, herbs and spices may contribute to the reduction of inflammation and be preventive against related diseases.

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