INTRODUCTION

Inflammation, a multi-step process, is mediated by activated inflammatory or immune cells. If not controlled, inflammation can lead to the development of such diseases as chronic asthma, rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease [1]. Macrophages play a central role in this process by mediating the immunopathological changes, including the overproduction of pro-inflammatory cytokines and mediators induced by activated tumor necrosis factor α (TNF-α) and cyclooxygenase 2 (COX-2) [2, 3]. The activation of macrophages is mediated by the activation of pattern recognition receptors (e.g., Toll-like receptor 4; TLR 4) by their ligands (e.g., lipopolysaccharide; LPS), which are released from bacteria or viruses [4]. TNF-α is a toxic cytokine involved in inflammation and other pathological processes, such as rheumatoid arthritis and microbial infections [5, 6]. COX-2 is an inducible immediate-early gene product in inflammatory cells and immune cells; it is mostly involved in chronic inflammatory processes and is markedly stimulated by LPS, cytokines, growth factors, and tumor promoters [7]. COX-2 produces prostaglandins (PGs) that contribute to the pain and swelling of inflammation [8, 9]. Both TNF-α and COX-2 are essential to the inflammatory response to pathogenic germs or toxicants. Thus, the suppression of these mediators may be an effective therapeutic strategy for preventing inflammatory reactions and diseases [10]. However, the adverse effects of some nonsteroidal anti-inflammatory drugs have stimulated an interest in identifying natural products for the prevention and treatment of inflammatory disorders [11]. Herbs and spices are extensively used in traditional medicine to relieve the symptoms of inflammatory disorders. Indeed, in vitro and in vivo studies have shown that diverse nonnutritive dietary compounds, such as curcumin, diallyl sulfide, capsaicin, eugenol, and gingerol, that are present in herbs and spices suppress the expression of pro-inflammatory gene products, including cytokines, chemokines, adhesion factors, and enzymes [10, 12, 13]. Several biologically active food additives have been developed (e.g., Antiartrol, Bambuflex, Dokholodan, and Joint Flex) by Russian firms (see the Federal Register of Biologically Active Food Additives, Moscow, 2000, Chapter 10). To date, very few anti-inflammatory drugs of herbal origin have been identified; however, a number of plants from ethnomedicinal databases are under laboratory investigation worldwide [14].

Here, we describe the screening of the anti-inflammatory activities of the extracts of 133 plants and mushrooms available from Russian suppliers (105 dried plants and 2 mushrooms) and supermarkets (26 vegetables and fruits).

LPS-stimulated human differentiated acute monocytic leukemia THP-1 cells have been used as a cell model to study the anti-inflammatory potencies of plant ethanolic extracts. The results demonstrate that non-cytotoxic quantities of several extracts attenuated the LPS-mediated TNF-α and prostaglandin E2 (PGE2) production in THP-1 cells.
EXPERIMENTAL CONDITIONS

Plant materials. Whole plants and plant parts were purchased from Moscow drugstores or supermarkets or were obtained from official suppliers as ready-to-use dried herbal preparations in 2009 (see table). The materials were collected within the territory of the Russian Federation from various regions, depending on the supplier. Dr. V. Karandashev identified the plant materials. Voucher specimens have been deposited in our laboratory at the Ajinomoto-Genetika Research Institute (ZAO AGRI). The herbal materials were stored in a dry, dark location under standard laboratory conditions.

Preparation of plant extracts. The preparation of the plant extracts was carried out by Dr. I. Malfanov and Dr. S. Ivanov (Ajinomoto-Genetika Research Institute), and the dried plant material was prepared as previously described [15]. Briefly, approximately 10 g of each plant was dried and crushed in a mill (if necessary) and soaked in 70% aqueous ethanol (1 : 40, w/v) with stirring (200 rpm) overnight at room temperature. The suspensions were filtered through filter paper (Whatman no. 4), and the ethanol was removed using a rotary evaporator. After freeze-drying, the crude extracts that were obtained had a mean yield of approximately 31%, ranging from 3.7% for Salsola collina to 94.1% for Beta vulgaris. The extracts were stored at –20°C in the dark until further analysis. Prior to the analysis, the dried extracts were solubilized in dimethyl sulfoxide (DMSO)/complete RPMI-1640 medium (1 : 100 v/v) at concentrations of 10.0, 1.0, and 0.1 mg/mL before the final dilution (1 : 9) with culture medium.

Chemicals and equipment. The nutrient medium (RPMI-1640), glutamine, penicillin, and streptomycin were obtained from NPP PanEko (Russia). The reactions were performed in 96-well flat-bottom clear polystyrene microplates (Corning, USA) with the aid of a Thermostatic Shaker ST-3 (Elmi, Latvia). The optical density (at 450 nm for the TNF-α and PGE2 assays and 540 nm for the MTT assay) was measured using a microtiter plate reader, Multiskan Ascent (Thermo Electron, USA).

Growth and activation of cells. Human THP-1 monocytic leukemia cells [16] were routinely maintained in RPMI-1640 medium containing with 10% FBS (HyClone, USA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. To reduce the risk of contamination of the nutrient medium with endotoxins, the FBS was heated at 56°C for 30 min and filter-sterilized before application. Freshly thawed THP-1 cells were cultured for each set of experiments and were not used for more than a few (3–5) passages. The cells were seeded at $2 \times 10^5$ cell/mL (4 $\times 10^4$ cell/well) and 8 $\times 10^4$ cell/mL (16 $\times 10^4$ cell/well) for the TNF-α and PGE2 assays, respectively, and were grown in 96-well plates sealed with a gas-permeable Breathe–Easy membrane BEM-1 (Diversified Biotech, USA) at 37°C under 5% CO2.

The differentiation of the THP-1 monocytes into macrophage-like cells was carried out with phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich, USA) applied at 10 ng/mL (16 nM), a concentration sufficient to induce stable cell differentiation without undesirable gene upregulation [17]. PMA was prepared as a stock solution (10 mg/mL) in DMSO and then diluted with complete RPMI-1640 medium to obtain the final concentrations. The cells were incubated in the presence of PMA for 24 h.

TNF-α assay. LPS from E. coli O127:B8 (Sigma-Aldrich, USA) was used to induce an inflammatory response in the differentiated THP-1 macrophages. The cells were grown in the presence of 12.5 ng/mL LPS in complete RPMI-1640 medium plus either 0.01, 0.1, or 1.0 mg/mL of the plant extracts for 4 h. The medium was then collected for the determination of the TNF-α level, and the cells were washed and incubated for 40 min with fresh medium containing 0.1 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Alfa Aesar, USA) to evaluate the cytotoxic effects of the plant extracts. The absorbance was measured at 540 nm, and the cell viability (percent of the control) was calculated relative to the untreated control. A TNF-α synthesis inhibitor, dexamethasone (Sigma-Aldrich, USA), was applied at 10 μM, and these samples served as positive controls. The amount of TNF-α was determined with a Human TNF-α ELISA Ready–SET–Go! kit (eBioscience, USA) according to the manufacturer’s instructions.

PGE2 assay. Differentiated THP-1 cells were grown in the presence of 1250 ng/mL LPS in complete RPMI-1640 medium plus 0.01, 0.1, or 1 mg/mL of the plant extracts for 24 h. The medium was then collected for the PGE2 analysis, and the cells were washed and incubated for 40 min with fresh medium containing 0.1 mg/mL MTT to evaluate the cytotoxic effects of the plant extracts. The COX-2-specific inhibitor nimesulide (Sigma-Aldrich, USA) was used as a positive control to suppress the COX-2 activity and, therefore, to inhibit PGE2 synthesis and release by the differentiated THP-1 macrophages. The nimesulide was dissolved in DMSO and applied at 5–10 μM with a final DMSO concentration ≤0.1%. The PGE2 production was measured using a R&D Systems Parameter PGE2 kit (R&D Systems, USA) (1 : 2 sample dilution, the high-sensitivity assay option) according to the manufacturer’s recommendations.

RESULTS AND DISCUSSION

The anti-inflammatory activities of the extracts of 105 plants used in Russian traditional medicine, 26 vegetables and fruits and 2 mushrooms were determined using LPS-stimulated differentiated human acute monocytic leukemia THP-1 cells as a cell model. The effects of these extracts were compared...