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Immunomodulatory properties of various natural compounds and essential oils through modulation of human cellular immune response

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ABSTRACT

Randomly selected essential oils and some terpene and phenolic derivatives were evaluated for their ability to modulate human cellular immune responses using various inflammatory parameters including reactive oxygen species (ROS) generation from whole blood phagocytes and isolated polymorphonuclear neutrophils (PMNs), proliferation of T-cells, IL-2. and TNF-α cytokine production. The essential oils from *Foeniculum vulgare*, *Saturea cuneifolia*, and *Origanum munitiflorum* inhibited ROS produced from whole blood phagocytes, while thymol, carvacrol, rosmarinic acid, caffeic acid, quercetin, and kaempferol glycoside potently inhibited the ROS production from whole blood as well as from isolated PMNs, among which caffeic acid, quercetin, and kaempferol glycoside exhibited a better inhibitory effect than that of ibuprofen. The compounds rosmarinic acid, caffeic acid, quercetin, kaempferol glycoside, genistein, and apigenin (IC_{50} 7.0 ± 0.5, <3.12, <3.12, 4.3 ± 1.0, 5.8 ± 0.5, and <3.12 μg/mL) were found to exert potent inhibition on proliferation of T-cells. On the other hand, quercetin (IC_{50} 2.9 ± 0.3 μg/mL) and apigenin (IC_{50} <1.0 μg/mL), were also found to be the potent inhibitor of IL-2 cytokine. The essential oils of *Mentha* species, and compounds quercetin and apigenin were found to potently inhibit the production of pro-inflammatory cytokine TNF-α. The results indicated that essential oils and phenolics might be considered as promising immunomodulatory agents.

1. Introduction

Recently, there has been a great increase of interest in phytochemicals having anti-inflammatory potential, since they are viewed as promising therapeutic agents due to their impact on the status of human health and disease prevention. Immune system is directly related to human health as its function is to prevent from foreign attacks to the body. The deregulation of immune response is strongly associated with various inflammatory or degenerative disorders including chronic and acute infections.

In inflammatory process, the various cells of the immune system including macrophages, neutrophils, and lymphocytes, releases mediators such as reactive oxygen species (ROS) and pro-inflammatory cytokines, *etc*. These mediators of innate immune response aid in propagation of inflammatory processes and activation of adaptive immunity. In early events of adaptive immune responses, $CD4^+$ T-helper cells activate various other type of cells, *i.e*., synovial fibroblasts, monocytes, and B-cells by releasing various cytokines among which TNF-α, IL-1β, and IL-2 play main role in progression of inflammation and, hence, can lead to various inflammatory and autoimmune diseases including rheumatoid arthritis, ankylosing spondilytis, psoriasis, atherosclerosis, type-2 diabetes, and Alzheimer's disease (Bradley, 2008; Lue et al., 2012).

Macrophages forming the first barrier in defense system of the body usually produce and secrete some pro-inflammatory cytokines, *i.e*., tumor necrosis factor (TNF)-α and interleukins (IL) which are used in determination of immune response in macrophage cell-based models (Stow et al., 2014). As one of the critical factors of immune response, TNF-α is well-known to be a fundamental mediator of various pharmacological activities including cell proliferation, cell death, and differentiation as well as

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initiation of inflammation and immune modulation (Al-Lamki and Mayadas, 2015).

Essential oils with a wide range of applications in the pharmaceutical, food, and cosmetic industries have gained a great popularity due to their various pharmacological effects desired for human health and many studies dealing with immunomodulatory effect of essential oils have been reported up to date (Saad et al., 2013). Moreover, monoterpenes, present commonly in the chemical compositions of essential oils, have exerted strong immunobiological properties through TNF-α, ILs, tromboxanes, and leukotrienes (De Cássia Da Silveira E Sá et al., 2013). In our previous research (Orhan et al., 2011), we reported a strong antimicrobial activity of several essential oils and their common pure components against 10 isolated strains of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamase (ESBL) enzyme, which is responsible for resistance against antibiotics by these strains. Consequently, the positive results obtained from that study with some selected essential oils (*Foeniculum vulgare*, *Satureja cuneifolia*, *Mentha* × *piperita*, *Mentha spicata*, *Origanum onites*, *Origanum munitiflorum*, *Origanum majorana*, and *Origanum vulgare* var. *hirtum*) and several compounds, some of which are generally found in essential oils (citronellol, thymol, vanillin, *iso*-borneol, menthol, borneol, carvacrol, and menthofurane) prompted us to investigate their immunomodulatory activity. Furthermore immunomodulatory properties of various other natural compounds of polyphenolic origin, *i.e.*, rosmarinic, quinic, and caffeic acids, quercetin, kaempferol-3-*O*-β-D-galactoside, silibinin, genistein, and apigenin were also assessed in this study.

2. Materials and methods

2.1. Plant materials

F. vulgare, *S. cuneifolia*, *M.* × *piperita*, *M. spicata*, *O. onites*, *O. munitiflorum*, *O. majorana*, and *O. vulgare* var. *hirtum* were cultivated in the experimental farm belonging to Faculty of Agriculture, Selcuk University, in ecological conditions of Konya province (Turkey) with a cold semi-arid steppe climate and less rainfalls (rain index 18.08), where the average temperature is around 11.3 °C. The city of Konya is located at between 36.5–39.58 north latitude and 31.5–34.58 east longitude and is the largest province of Turkey with a surface area of 38,183 km^2. The plant samples were harvested at fully mature stages of the plants during the harvest season in 2008, dried in shade immediately, and stored at +4 °C prior to the experiments.

2.2. Tested compounds

The monoterpenes citronellol, *iso*-borneol, menthol, borneol, carvacrol, and menthofurane, the aromatics, *i.e.*, thymol, vanillin, the polyphenolics rosmarinic, quinic, and caffeic acids, quercetin, silibinin, genistein, and apigenin were purchased from Carl Roth Chemical Company (Karlsruhe, Germany). Kaempferol-3-*O*-β-D-galactoside was earlier isolated from *Calluna vulgaris* by our group as earlier described in our paper (Orhan et al., 2007).

2.3. Distillation of the essential oils

The dried aerial parts (100 g for each) of each plant species were subjected to hydrodistillation for 3 h using Clevenger-type apparatus to obtain the essential oils studied herein. The essential oil yields (v/w%) were 1.2% for *F. vulgare*, 1.0% for *S. cuneifolia*, 3.2% for *M.* × *piperita*, 1.8% for *M. spicata*, 2.6% for *O. onites*, 3.1% for *O. munitiflorum*, 3.5% for *O. majorona*, and 2.8% for *O. vulgare* var. *hirtum*. The essential oils were stored at −20 °C until experimental studies.

2.4. Immunomodulatory assays

2.4.1. Reagents, chemicals, and equipments

Luminol (3-aminophthalhydrazide), Hanks Balance Salts Solution (HBSS), and Lymphocytes Separation Medium (LSM) were purchased from Research Organics (Sigma, Germany) and MP Biomedicals Inc. (Germany), respectively. Zymosan-A (*Saccharomyces cerevisiae* origin), dimethylsulfoxide (DMSO), ethanol, and ammonium chloride of analytical grade were purchased from Merck Chemicals (Darmstadt, Germany). The luminometer used was Luminoskan RS (Finland).

2.4.2. Isolation of human polymorphonuclear cells (PMNs)

Heparinized blood was obtained by vein puncture aseptically from healthy volunteers (age of 25–38 years). The PMNs were isolated by Ficoll Hypaque density gradient centrifugation from the tube base. Cells were washed twice and suspended in Hank's Balance Salt Solution (Ca^{2+} and Mg^{2+} free) ($HBSS^{--}$), pH 7.4. Neutrophils were purified from RBCs (red blood cells) contamination using hypotonic solution. Cells were adjusted to their required concentration using Hank's Balance Salt Solution containing Ca^{2+} and Mg^{2+} ($HBSS^{++}$).

2.4.3. Chemiluminescense assay for oxidative burst study

Luminol-enhanced chemiluminescence assay was performed as described by Yamamura et al. (1992) with some modifications. Briefly, 25 μL diluted whole blood (1:200 dilutions in sterile $HBSS^{++}$) or 25 μL of PMNs (1×10^6) cells were incubated with 25 μL of serially diluted test samples with concentration ranges between 3.2 and 50 μg/mL. Tests were performed in white 96-well plates, which were incubated at 37 °C for 30 min in the thermostatic chamber of the luminometer. Opsonized zymosan-A, 25 μL followed by 25 μL luminol (7×10^{-5} M) along with $HBSS^{++}$ was added to each well to obtain a 100 μL volume/well. Wells received $HBSS^{++}$ and cells but no compounds were used as a negative control. Results were monitored as relative light units (RLU) with peak and total integral values. Percent inhibition values were calculated using the following formula. These values were then used for calculating IC_{50} (inhibitory concentration) using Excel-based formula, *i.e.*, the concentration that inhibits 50% of ROS produced.

$$\%\text{Inhibition} = \frac{100 - \text{mean reading of test compound}}{\text{mean reading of positive control}} \times 100$$

2.4.4. T-cell proliferation assay

Cell proliferation was measured by standard thymidine incorporation assay (Mesaik et al., 2012). Briefly, cells were obtained from the peripheral blood of healthy individuals and then cultured at a concentration of 2×10^6 cells/mL in a 96-well round-bottom tissue culture plates. Cells were stimulated with 5 μg/mL phytohemagglutinin (PHA). Five different concentrations of the samples in triplicate were added, which ranged between 3.1 and 50 μg/mL. The plate was incubated for 72 h at 37 °C in 5% CO_2 incubator. After 72 h, cultures were pulsed with 0.5 μCi/well-tritiated thymidine and further incubated for 18 h. Cells were harvested onto a glass fiber filter using cell harvester. The level of the thymidine incorporated into the cells was measured by a liquid scintillation counter. Results were expressed as mean count per minute (CPM). The inhibitory activity of the samples on T-lymphocyte proliferation was calculated using the following formula:

$$\text{Inhibitory activity}(\%) = \frac{\text{control group(CPM)} - \text{experiment group(CPM)}}{\text{control group(CPM)}} \times 100$$

2.4.5. IL-2 cytokine assay

IL-2 cytokine was produced from peripheral blood mononuclear cells (PBMC). IL-2 cytokine production by PHA-activated cells in the presence or absence of test samples was studied by ELISA using the human cytokine kit (Diaclone, Besancon Cedex France).