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Chemical compositions and anti-inflammatory activities of essential oils from *Aster spathulifolius* and *Vitex rotundifolia* maxim

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ABSTRACT

The essential oils were prepared from *Aster spathulifolius* (ASE) and *Vitex rotundifolia* (VRE) by hydrodistillation and their chemical compositions were investigated by GC–MS. Analysis of ASE provided 15 components and eight of which were identified sesquiterpene compounds. The major components in ASE included germacrene D (35.1 %), trans-caryophyllene (15.9 %) and trans-phytol (14.9 %). On the other hand, VRE provided manoyl oxide (14.3%), α-terpineol (13.1 %) and α-pinene (10.0 %) as major ingredients. To assess the anti-inflammatory effects of the essential oils, the production of nitric oxide (NO) and tumor necrosis factor (TNF)-α were monitored using lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. In this test, ASE and VRE were appeared to suppress both NO and TNF-α synthesis in dose-dependent manner. The results indicate that VRE and ASE could be useful in cosmetic applications as natural products possessing anti-inflammatory efficacy.

INTRODUCTION

The essential oils are mixture of volatile compounds possessing aromatic odours derived from mainly plant flowers or leaves. Terpenoids, isoprene-based natural compounds, are in most cases the major chemical components in the essential oils. Identification of the individual chemical constituents in the oils could be accomplished by the use of gas chromatography-mass spectrometry (GC-MS), where the relative retention times/indices and fragmentation patterns were comparatively analyzed for the specific GC peaks (Zellner *et al*., 2010). Essential oils have been commonly applied in aromatherapy where the aromatic compounds are expected to have some curative effects. In order to identify new biological benefits in human healthcare, essential oils have been investigated in broad range of applications (Buchbauer 2010). We are continuously conducting research projects on essential oils prepared from plants in Jeju, an island of biodiversity located at the southernmost part of Korea (Kim *et al*., 2013; Kim *et al*., 2011). In this study, essential oils from *Aster spathulifolius* (ASE) and *Vitex rotundifolia* (VRE) by hydrodistillation were investigated for their chemical compositions along with their anti-inflammatory activities.

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A. spathulifolius Maxim. (Asteraceae) is a herb distributed in southern part of Japan and Korea. *V. rotundifolia* belonging to Lamiaceae is a woody shrub native to seashore throughout the Pacific. Both perennial plants commonly occur in the coastal area of Jeju Island (Lee *et al*., 2001). Antiviral efficacies have been studied on the extract from *A. spathulifolius* against the influenza infection (Won *et al*., 2013). Diterpenes with cytotoxic properties have been isolated from the aerial part of *A. spathulifolius* (Lee *et al*., 2005). Inhibition effects of the extract of *V. rotundifolia* on inflammatory gene expression in human epithelial cells have been reported (Sohn *et al*., 2009).

While chemical composition of essential oil from *V. rotundifolia* by distillation in the co-presence of water and petroleum ether has been analyzed (Jang *et al*., 2000), anti-inflammatory effects of VRE have not been explored yet.

MATERIALS AND METHODS

Plant material

The plants *A. spathulifolius* (sample no. 323) and *V. rotundifolia* (sample no. 324) were collected at the coastal area of Jeju Island during November and December in 2012. Voucher specimens have been deposited at the Natural Product Chemistry Laboratory, Jeju National University. The specimens were identified by Dr. H. C. Kim (plant taxonomist).

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Extraction of essential oils

The leaves of *A. spathulifolius* and the stems of *V. rotundifolia* were used as the materials for thisexperiments. Both samples were freeze-dried, and were subjected to water distillation for 6 h using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate, and were stored in a sealed vial at 4 °C during the experiment. The obtained oil yields were about 0.12% and 0.05% (w/w) for *A. spathulifolius* and *V. rotundifolia* respectively.

Gas chromatography-mass spectrometry analysis

Gas chromatographic analyses were performed on a Shimatzu GC-MS ultra 2010 gas chromatograph equipped with an non-polar Rtx-5MS column (30 m × 0.25 mm 0.25 μm) and a split-splitless injection port (split mode). The temperature was set at 40 °C for 5 min, ramped to 100 °C at 10 °C/min and further to 300 °C at 2 °C/min, and maintained at 300 °C for 20 min. Compounds were identified by their retention indices on the column and by GC-MS library. Results of the analysis for ASE and VRE were given in Tables 1 and 2.

Cell culture and viability

Murine RAW 264.7 macrophages were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) in an incubator at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

RAW 264.7 cells were cultured in 96-well plates for 18 h, followed by treatment with various concentrations of the ASE and VRE. After 24 h incubation, MTT was added to the medium for 4 h. After removing the supernatant, formazan crystals were dissolved in DMSO and absorbance was measured at 540 nm. The percentage of cells showing cytotoxicity was determined relative to the control group.

Determination of nitric oxide (NO) products

After pre-incubation of RAW 264.7 cells (2.0×10^5 cells/mL) with LPS (1 μg/mL) for 24 hours, the quantity of nitrite in the culture medium was measured as an indicator of NO production. Briefly, 100 μL of cell culture medium was mixed with 100 μL of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid), the mixture with different concentration (3.13, 6.25, 12.5 and 25.0 μg/mL) of oils was incubated at room temperature for 10 min, and the absorbance at 540 nm was measured in a microplate reader. Fresh culture medium was used as a blank in every experiment. The quantity of nitrite was determined from a sodium nitrite standard curve.

Measurement of Pro-inflammatory Cytokine (TNF-α) Production

The inhibitory effects of ASE and VRE on pro-inflammatory cytokine (TNF-α) production in LPS-treated RAW 264.7 cells were determined by ELISA as described in the manufacturer's instructions (R & D Systems, Minneapolis, MN).

RESULTS AND DISCUSSION

Water-distilled essential oils from the leaves of *A. spathulifolius* (ASE) and the stems of *V. rotundifolia* (VRE) were analyzed by GC and GC-MS. The identified compounds with their percentage were summarized in Tables 1 and 2. The GC/MS retention indices were calculated using a homologous series of C_5-C_{26} *normal*-alkanes. Analysis of ASE afforded fifteen components representing 100% of the total oil (Table 1). A cyclic sesquiterpene, germacrene D (35.1%), was identified as the major component. The other important ingredients include *trans*-caryophyllene (15.9%), *trans*-phytol (14.9%), (-)-caryophyllene oxide (6.0%) and 6,10,14-trimethyl-2-pentadecanone (5.6%). ASE is characterized by high contents of sesquiterpene and diterpene compounds.

Table 1: Composition of essential oil from *A. spathulifolius* (ASE).

RT	RI	Name	%
32.9	1418	*trans*-Caryophyllene	15.9
34.5	1459	(Z)-β-farnesene	1.2
35.4	1483	Germacrene D	35.1
35.9	1495	bicyclogermacrene	4.1
36.7	1529	Methyl (Z)-dec-2-en-4,6-diyonate	2.0
36.8	1537	δ-Cadinene	2.2
38.5	1641	Spathulenol	1.1
38.6	1655	(-)-Caryophyllene oxide	6.0
40.6	1831	α-Cadinol	1.2
41.9	1920	Stearaldehyde	1.4
43.1	1923	2-(1-E-propenyl)-4-methoxyphenyl 2-methyl butanoate	4.3
44.7	1948	Neophytadiene	1.4
44.9	1951	6,10,14-Trimethyl-2-pentadecanone	5.6
49.0	2096	α-Podocarpene	3.5
50.2	2263	*trans*-phytol	14.9
Total			100

In the oil of *V. rotundifolia* (VRE), twelve components were verified through a typical library search selecting only components with matches exceeding 80%, which represented about 70.7% of total oil (Table 2). The major components detected in VRE were manoyl oxide (14.3%), α-terpineol (13.1%), α-pinene (10.0%), dehydroabietane (5.9%) and sabinene (5.1%). As seen in Table 2, VRE consisted mainly of mono- and diterpenes. Previous report on the composition of oil of *V. rotundifolia* has indicated its major components as α-pinene (13.2%), α-terpineol (10.6%), 1,8-cineole (4.4%) and manoyl oxide (4.0%). Comparing our results to the literature though the compositions for both oils are almost identical but the percentage of each component showed so report (Jang *et al*., 2000), even me discrepancies. This could be attributed to the difference of the distillation method between this study and the literature one.

Table 2: Composition of essential oil from *V. rotundifolia* (VRE).

RT	RI	Name	%
10.4	932	α-Pinene	10.0
11.9	973	Sabinene	5.1
11.9	974	2-β-Pinene	3.1
13.9	1029	1,8-Cineole	4.9
19.5	1188	1-Methyl-4-(2-propanol-2-yl)-cyclohexene	1.9
29.6	1357	α-Terpineol	13.1
47.2	1987	β-*iso*-Methyl ionone	2.2
47.3	1988	Biformene	4.3
47.5	1992	Manoyl oxide	14.3
49.3	2128	Dehydroabietane	5.9
50.2	2256	*trans*-Phytol	3.6
50.4	2309	Thunbergol	2.3
Total			70.7

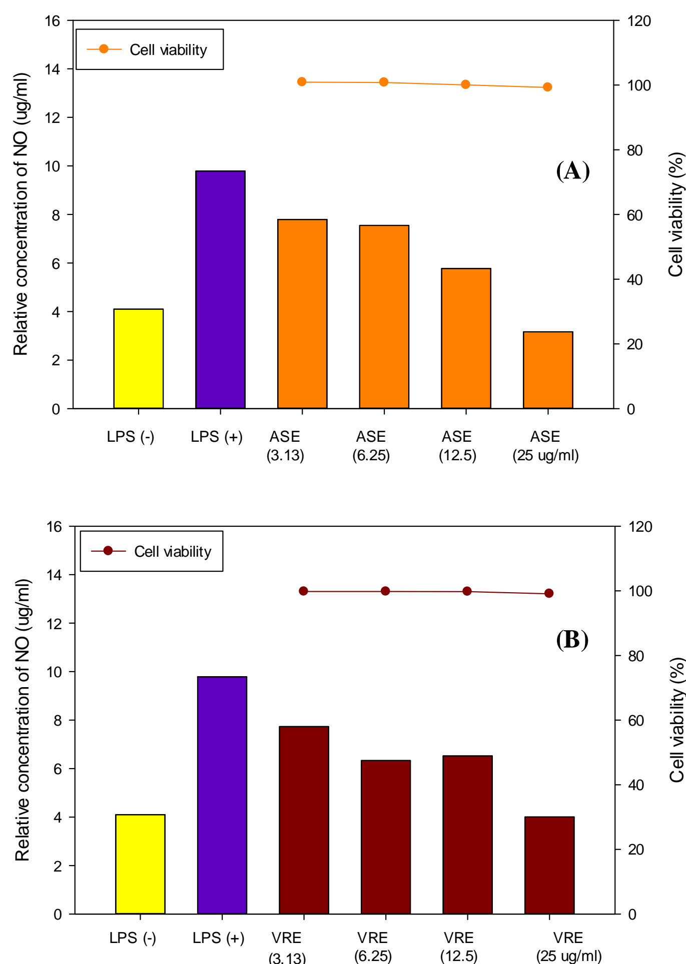

Fig 1. Nitric oxide (NO) production and cell viability for ASE (A) and VRE (B) in RAW 264.7 cells. RAW 264.7 cells (2.0×10^4 µg/mL) were pre-incubated with LPS for 24 h, and nitrite was assayed after treatment of different concentration (3.13, 6.25, 12.5 and 25.0 µg/mL) of oils. The absorbance was measured at 540 nm with a spectrophotometer (Power Wave; Bio-tek, Winooski, VT).

In order to validate the use of oils as an anti-inflammatory agent, their effects on the production of nitric oxide (NO) was investigated (Yoon *et al*., 2010). Nitric oxide is an endogenous free radical species synthesised from L-arginine by nitric oxide synthase (NOS) in various animal cells and tissues. After exposure to triggers including lipopolysaccharide (LPS),

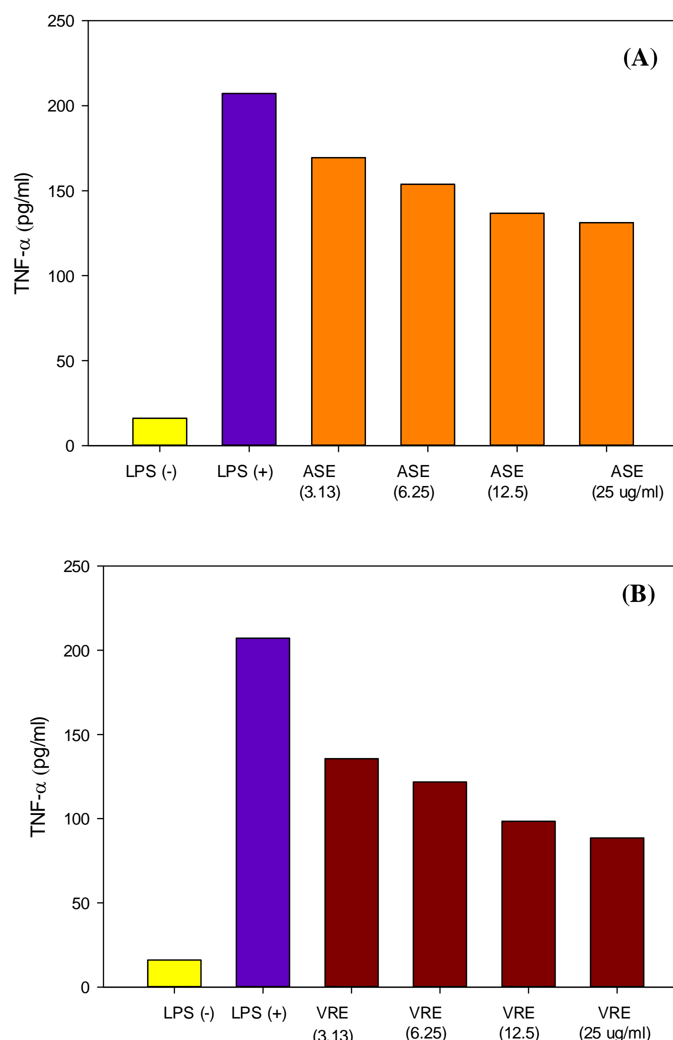

Fig 2. Effects of ASE (A) and VRE (B) on TNF-α production in LPS-stimulated RAW 264.7 cells. TNF-α production in LPS-treated RAW 264.7 cells were determined by ELISA after treatment of different concentration (3.13, 6.25, 12.5 and 25.0 µg/mL) of oils as described in the manufacturer's instructions (R & D Systems, Minneapolis, MN).

inducible NOS (iNOS) can be generated in inflammation-related cells such as macrophages (Murakami 2009).

Thus, measuring NO production may be a useful method for assessing the anti-inflammatory effects of essential oils. In this experiment, the nitrite concentration was determined in the supernatant after treatment with LPS (1 µg/mL) alone or co-treated with ASE and VRE (3.13 to 25 µg/mL) for 24 h using Griess reagent. As shown in Fig. 1, ASE and VRE suppressed LPS-induced NO formation significantly in dose-dependent fashion. ASE and VRE exhibited similar potencies in this assay providing about 60% decrease of NO production at 25.0 µg/mL concentration.

In order to inspect the oils' cytotoxicity, cell viability was assessed using an MTT assay. As shown in the same figure, the numbers of viable macrophage cells were rarely decreased by the ASE and VRE indicating their non-toxic properties. This indicated that the inhibitory effects by the oils were not due to

cytotoxicity or cell death, but were derived by other anti-inflammatory mechanisms. TNF-α is a cytokine primarily produced by monocytes and macrophages eliciting septic shock and inflammation. The inhibition of TNF-α production or function is a key mechanism in the control of inflammation (Feldmann 2008). Levels of TNF-α in the culture supernatants were measured using ELISA kits. LPS (1 μg/mL) stimulation for 24 h led to marked increases of TNF-α levels in the cell supernatants. However, treatment ASE and VRE for 24 h exhibited a concentration-dependent inhibition of TNF-α production in RAW 264.7 cells (Fig. 2).

In this test, VRE exhibited relatively potent activities compared to ASE. In summary, essential oils ASE and VRE were chemically analyzed by GC-MS and their major components were identified terpenoids including germacrene D and manoyl oxide respectively. The anti-inflammatory effects were monitored by the production of nitric oxide (NO) and TNF-α using RAW 264.7 macrophages. In this test, ASE and VRE were appeared to suppress both NO and TNF-α synthesis in dose-dependent manner. The results indicate that VRE and ASE could be useful in cosmetic applications such as aromatherapy as natural products possessing anti-inflammatory efficacy.

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