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THE EFFECT OF ANTIOXIDANT ACTIVITIES (DPPH, NO, ROS) BY BLEND ESSENTIAL OILS

Sang Nam Park[1], Yong Lim[2], Un Kyu Park[1], Man Kyu Huh[3]* and Seock-Yeon Hwang[1]*

[1]Department of Biomedical Laboratory Science, Daejeon University, Daejeon 34520, S. Korea.
[2]Department of Clinical Laboratory Science, Dong-eui University, Busan 47340, S. Korea.
[3]Food Science & Technology Major, Dong-eui University, Busan 47340, S. Korea.

***Corresponding Author: Seock-Yeon Hwang, Man Kyu Huh**

Department of Biomedical Laboratory Science, Daejeon University, Daejeon 34520, S. Korea.

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ABSTRACT

The antioxidant activity of four blend oils from limonene oil, lavender oil, pepper mint oil, eucalyptus oil, and tea tree oil was measured by the inhibition of 1, 1- diphenyl 2-picrylhyorazyl (DPPH), nitric oxide (NO), and reactive oxygen species (ROS). All the oils showed about 90% DPPH inhibitory scavenging ability. Blending oils showed a decrease of 20% and 30% NO inhibitory scavenging ability at concentrations of 0.001 and 0.01‰, respectively. Three blending oils did not show a significant decrease of LPS-induced ROS generation. Tea tree oil and eucalyptus oil had higher activity on DPPH and NO than the corresponding other oils. Tea tree oil showed the strongest ROS activity among the oil products tested. The antioxidant activity of the samples seems to be based on their free radical scavenging capacity. The inhibitory activity was higher when the complex (blend) substance was treated than the single substance.

KEYWORDS: antioxidant activity, 1, 1- diphenyl 2-picrylhyorazyl (DPPH), nitric oxide (NO), reactive oxygen species (ROS).

INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Many natural compounds extracted from plants have important biological activities. Among these compounds, an essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. They are what give the plant its characteristic odor and contain the healing power of the plant from which it was extracted. Essential oils have grown in popularity during recent years for good reason. When used correctly, essential oils bring a wide range of health benefits since unlike modern drugs.

Essential oil has various physiological activities.[1] Many authors, in fact, have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties by spices and essential oils.[2-3] Essential oil's antioxidant activity has the mechanism that essential oil component electronically stabilizes free radical of 1, 1- diphenyl 2-picrylhyorazyl (DPPH) by giving up radical or hydrogen radical or donating electron to reduce toxicity of free radical.[4] Lin et al.[5] evaluated the antioxidant activities of the methanolic extract of many species, which was found to be potent in free radical scavenging activity especially against DPPH radicals. Active oxygen, DPPH radical scavenging effect, nitrogen monoxide inhibition and lipid peroxidation were evaluated in the combination of four essential oils.[6] Essential oils have been widely used for their virucidal, bactericidal, fungicidal, anticancer, antioxidant, antidiabetic activities.[7] The essential oil of *Casearia sylvestris* presents a good selective cytotoxicity against HeLa, A-549 and HT-29 tumor cells when compared to non-tumoral cells Vero and mice macrophages.[8] Most reports of essential oils were determined by original extraction. These essential oils of original extraction were different from commercially used essential oils. Blending essential oils are mixtures of several components. The antimicrobial and antioxidant activities of these compounds are well known and have been reported in many studies.[9-11] DPPH is a common abbreviation for the organic chemical compound 1, 1- diphenyl 2-picrylhyorazyl or 2,2-diphenyl-1-picrylhydrazyl. The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals.[4] Nitric oxide (NO) and reactive nitrogenspecies (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species.[12] NO produced by a variety of mammalian cells and is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities.[13] Cells constantly generate reactive oxygen species (ROS) during aerobic metabolism. ROS scavenging activities have been shown to exhibit multiple biological effects, including antiallergic,

antibacterial, antidiabetic, anticancer and anti-inflammatory activities.[14-15]

In this study, four mixed Korean essential oils with different chemical compositions were studied, because of the great heterogeneity in the composition that exists between the plants of six species. The purpose of this study was to evaluate how the plant composition and biological activities which are both studied in this work, as well as how the antimicrobial activity, cytotoxicity, and inflammatory cytokine production is influenced by these changes.

MATERIALS AND METHODS

Materials

The blend oils as test substances were five kinds of the essential oil and supplied by Company B (Table 1).

Table. 1: The lists of four blending essential oils.

Essential oil	#1	#2	#3	#4
Limonene oil	40	40	40	40
Lavender oil	**30**	10	10	10
Pepper mint oil	10	**30**	10	10
Eucalyptus oil	10	10	**30**	10
Tea tree oil	10	10	10	**30**
Total	100	100	100	100

Preparation of sample: 9 mL samples of blend extracts in ethanol were separately added to a 1 mL solution of DPPH radical in ethanol (final concentration of DPPH was 10%). NO and ROS ethanolic solutions were prepared at a concentration of 0.01‰ and 0.001‰.

DPPH assay: The antioxidant activity of the blend essential oils was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al.[4] with slight modifications. DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 ul of ethanol DPPH solution (final concentration 300 μm) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes are measured at 517 nm. In the control group, distilled water was added instead of the sample solution, and ethanol was added instead of the DPPH solution to obtain a correction value.

The inhibition % was calculated using the following formula. Percentage inhibition was calculated using the following formula: % Inhibition = [1 - OD (DPPH + sample)/OD (DPPH)] x 100%.

Nitric oxide: The assay is the nitric oxide radical scavenging assay.[15] The extracts were prepared from a 10mg/mL ethanol crude extract. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5mL of 10mM sodium nitroprusside in phosphate buffered saline was mixed with 1mL of the different concentrations of the ethanol extracts and incubated at 25°C for 2 h. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. A volume of 150μL of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 540 nm using a UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded.

Measurement of ROS production in RAW264.7 cells using DCF-DA: 2', 7'-dichlorofluorescin diacetate (DCF-DA) was used to measure reactive oxygen species (ROS) in Raw 264.7 cells. Raw 264.7 cells were seeded at 5 x 10 cells / well in 2 well plates. After incubation for 4 hours, five kinds of essential oil were treated at a concentration of 0.1, 1 (‰) and 1 μg / ml of LPS, and cultured in an incubator (37 ºC, 5% CO_2) for 24 hours. After centrifugation, the collected cells were washed twice with cold PBS, added with DCF-DA 10 μm, and stained at room temperature for 15 minutes. After washing them with cold PBS, centrifugation was carried out at 1,200 rpm for 5 minutes. The supernatant was removed and 400 μl of PBS was added thereto. The fluorescence intensity was measured using a flow cytometer (BECTON Dickinson, Franklin Lakes, NJ, USA).

Statistical analysis: All the analysis were carried out in triplicate and the results were expressed as the mean ±SD. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0). The percent inhibition was calculated as the decolourization percentage of the test

sample using the following formula: Inhibition % = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells. The experimental results were statistically analyzed using student's t-test and significance was tested at P <0.05, P <0.01 and P <0.001.

RESULTS

DPPH scavenging ability

The DPPH free radical scavenging ability was confirmed at the concentration of 10% blending oil. All the oils showed about 90% DPPH inhibitory scavenging ability (Table 1). Tea tree oil and eucalyptus oil had higher activity on DPPH than the corresponding other oils. The inhibitory activity was higher when the complex (blend) substance was treated than the single substance (data not shown).

Table. 1: DPPH radical scavenger activity of blending oils.

Sample	Concentration (%)	Mean (%)
Blending oil 1	10	89.9±10.5
Blending oil 2	10	89.7±7.1
Blending oil 3	10	82.8±8.9
Blending oil 4	10	93.0±4.5

Production of nitric oxides induced by LPS: Blending oil 1 showed a decrease of 20% and 30% NO inhibitory scavenging ability at concentrations of 0.001 and 0.01 ‰, respectively (Fig. 1A). Blending oil 2 showed a decrease of 15% and 25% at concentrations of 0.001 and 0.01 ‰, respectively (Fig. 1B). In blending oil 3, both concentrations showed a decrease of 25% (Fig. 1C) and in blending oil 4, both concentrations showed a decrease of 30% (Fig. 1D). Tea tree oil and eucalyptus oil had higher activity on NO than the corresponding other oils. The inhibitory activity was higher when the complex (blend) substance was treated than the single substance (data not shown).

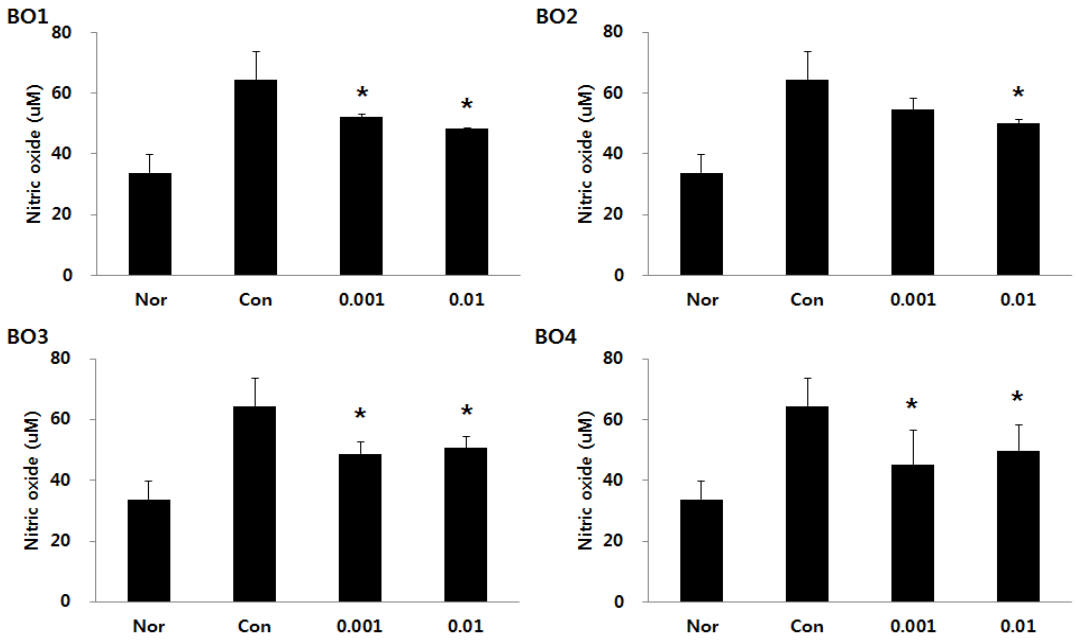

Figure 1. Effects of blending oils on LPS-induced NO production in Raw 264.7 cells. Raw 264.7 cells were treated with different concentrations of blending oils and LPS (1μg/Mℓ) for 24h. Each value represent the mean ± S.D. from 3 independent experiments. (BO1), blending oil number 1; (BO2), blending oil number 2, (BO3), blending oil number 3; (BO4), blending oil number 4.

LPS-induced ROS generation: Blending oil 1-3 did not show a significant decrease of LPS-induced ROS generation (Fig. 2A, B, C). In case 4, the concentration decreased by 10% at concentration of 0.01 ‰ (Fig. 2D). Tea tree oil showed the strongest ROS activity among the oil products tested. The inhibitory activity was higher when the complex (blend) substance was treated than the single substance (data not shown).

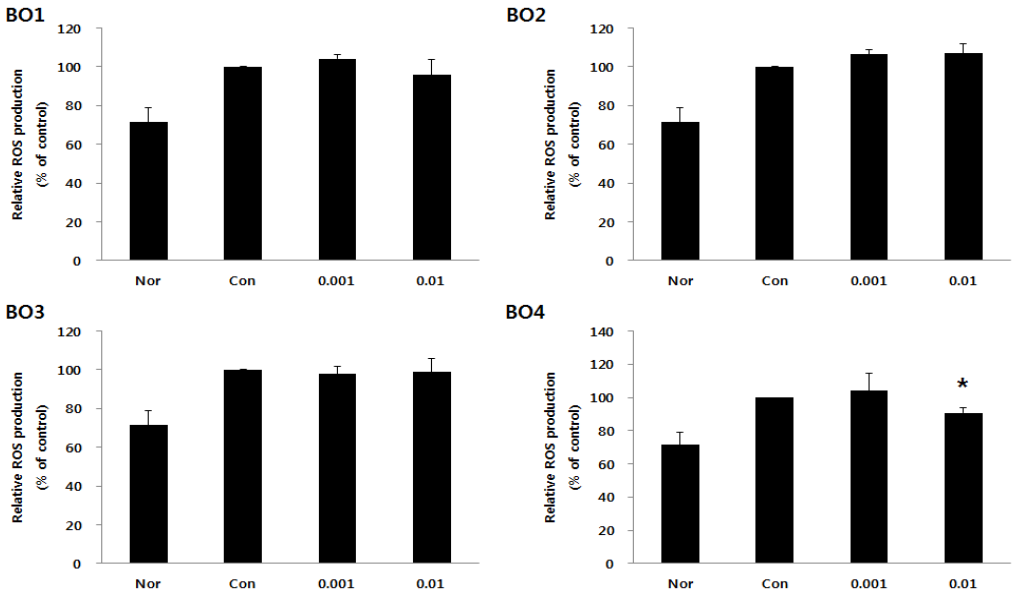

Figure. 2: Effect of blending oils on ROS product in RAW 264.7.

RAW 264.7 cells were treated with different concentrations of blending oils and LPS (1㎍/㎖) for 24h. Each value represent the mean ± S.D. from 3 independent experiments. (BO1) blending oil number 1; (BO2) blending oil number 2, (BO3) blending oil number 3; (BO4) blending oil number 4.

DISCUSSION

In DPPH method, the antioxidants react with the stable free radical. 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging activities of the sample/antioxidant and it has been found that the known antioxidants such as cysteine, glutathione, ascorbic acid, tocopherol and polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.) reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability.[16] In the present study, the blend oils of ethanolic extract were able to reduce the stable radical DPPH to 1,1-diphenyl-2-picrylhydrazine with 10%. NO can be quickly converted into a series of powerful oxidants with many biological effects by its diffusion-limited reactions with many free radicals.[17] The blend essential oil from limonene oil, lavender oil, pepper mint oil, eucalyptus oil, and tea tree oil was demonstrated to exhibit anti-inflammatory activity in Raw 264.7 cells.All of the essential oils that were shown to have significant antioxidant activities are produced from herbal plants that are commonly used as spices or as food flavoring additive, therefore, they are safe to use, yet provide good defense against oxidative damage and associated health effects.[18]

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