Antioxidant Activities and Analysis of Volatile Composition in the Leaves of *Ocimum basilicum*

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**ABSTRACT**: This study was designed to examine the antioxidant activity of the essential oil and ethanol extract of *Ocimum basilicum* (Lamiaceae). The ethanol extract was particularly found to possess strong antioxidant activity while essential oil showed moderate activity. GC/MS analyses of the oil resulted in the identification of 26 compounds, representing 99.98% of the oil; Linalool (37.13%), geraniol (8.91%), T-cardanol (7.76%), -bergamotene (7.73%), Eugenol (6.33%), 1,8-cineole (5.82%) and α-amorphene (4.76%) were the main components. The samples were also subjected to screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The free radical scavenging activity of ethanol extract was superior to essential oil (IC₅₀ = 20.28 g/ml).

**Key words**: *Ocimum basilicum*, Lamiaceae, Labiatae, basil, Essential oil, Antioxidant activity; GC-MS

The list of disorders in which free radicals are thought to contribute to their patho-physiology includes many chronic illnesses: inflammatory and immune diseases such as rheumatoid arthritis, ischemia-reperfusion, atherosclerosis, Alzheimer's disease, Parkinson's disease, radiation injury from UV and other ionizing radiation, certain forms of chemical carcinogenesis and cellular aging, to list a few (Cross, 1987). Accordingly, attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them (Larson, 1988).

The preservative effects of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues (Hirasa and Takemasa, 1998). Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids (Velioglu et al., 1998). Similarly, essential oils of many plant spices have demonstrated numerous biological activities, including antimicrobial and antioxidant activities (Carlton et al., 1992; Piccaglia et al., 1993; Buchbauer and Jirovetz, 1994; Aruna and Sivaramakrishnan, 1996; Jzet Dongmo et al., 2002). Many herb spices, especially those belonging to the Lamiaceae family, such as *Sage, Oregano, Mentha, Salvia* and *Thyme*, show strong antioxidant activity (Hirasa and Takemasa, 1998; Tepe et al., 2003; Marinova and Yanishlieva, 1997) and these herb spices is widely used in nutrition and is highly appreciated as a source of the essential oils (Lawrence, 1993). The essential oils of these species and of many other species of the Lamiaceae are mostly composed of mono- and sesquiterpenes (Lawrence, 1993). A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts (Nakatani, 1997). *Ocimum basilicum* L., (Lamiaceae) is also an important medicinal plant and culinary herb (Putievsky and Galambos, 1999). *O. basilicum* is a major essential oil crop with a remarkable infraspecific variation in plant morphology and oil composition (Lawrence, 1992). Several chemotypes have been established (Chalchat et al., 1999; Grayer et al., 1996; Marotti et al., 1996), those with either linalool or methyl chavicol or

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mixture of linalool and methyl chavicol or a mixture of linalool and eugenol or a mixture of methyl chavicol and methyl eugenol. In addition, essential oil of Ocimum basilicum possesses antifungal, insect repelling, antimicrobial and toxic activities (Revenet et al., 1984; Dube et al., 1989; Opalchenova and Obreshkova, 2003; Werner, 1995; Hili, 1997).

The aims of this study were conducted to find the antioxidant activity for utilization of crossing materials to improve natural sources using essential oil and herbal parts of O. basilicum. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical photometric assay in a process guided by its discoloration (Xiong et al., 1996).

MATERIALS AND METHODS

Essential oil and Plant material
The essential oil and herbal parts of O. basilicum were obtained from Nepal. A samples have been deposited at the Functional Bio-resources Laboratory of the College of Biological Resources and Technology of Dankook University (N67).

Extraction of Ocimum basilicum
The dried herbal parts of the O. basilicum was reduce to powder and extracted twice with ethanol at room temperature for 7 days and filtered. The combined ethanolic extracts were concentrated in vacuo.

Chemicals
All chemicals were used the highest grade available. Ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co. (St. Louis, MO). Ethanol and methanol of analytical grade were purchased from Merck (Darmstadt, Germany).

GC-MS analysis conditions
The analysis of the essential oil was performed using a Hewlett Packard 6890 GC, equipped with a HP-Innowax column (50 m×0.32 mm×0.5 m) and a HP 5973 mass selective detector. For GC-MS detection an electron ionization system with ionization voltage of 70 eV was used. Helium was the carrier gas, at a flow rate of 1.3 ml/min. Injector and MS transfer line temperatures were wet at 240 and 250°C, respectively. Column temperature was initially kept at 70°C, then gradually increased to 170°C at a 3°C/min rate and finally raised to 230°C at 10°C/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. Identification of the oil components were performed by GC-MS, comparing their retention times with those of pure substances by peak enrichment with standards. In some cases parallel analysis was carried out by Wileys library (HP 6890/HP 5873) in computer.

DPPH scavenging activity
Each Sample stock solutions (0.25 μg/ml) were diluted to final concentrations of 100, 50 and 25 μg/ml, in ethanol. 150 μl of 150 μM DPPH methanol solution was added to 100 μl of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using the following formula: AA % = 1 - (Abssample - Absblank)/(Abscontrol - Absblank) × 100. Ethanol (150 μl) plus plant extract solution (100 μl) was used as a blank. DPPH solution (150 μl; 150 μM) plus ethanol (100 l) was used as a negative control. The positive controls were those using the standard solutions.

The IC50 values were calculated by linear regression of the IC50 values were calculated by linear regression of the plots where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of antioxidant activity from three separate tests.

RESULTS AND DISCUSSION

Chemical composition of the essential oil
The composition of O. basilicum oil was analyzed by employing GC-MS, leading to compare the relative retention times and the ass spectra of oil components with those of authentic samples and mass spectra from data library.

As shown in Table 1, GC/MS analysis of the crude oil resulted in the identification of 26 compounds representing about 99.98% of the total oil. These yields were relatively higher than the average oil yields reported by Grayer et al. (1996) from the dried leaves of O. basilicum cultivars (99.2% of Italy, 94.3% of Holland, 95.9% of India, 97.1% of Yemen, 97.9% of U.S.A. and 90.7% of Brazil; flat leaves and 91.2-95.9% of U.S.A.; convex leaves). The essential oil of O. basilicum was characterized by a high number of monoterpenes. Linalool (37.13%), geraniol (8.91%), T-cardinal (7.76%), -bergamotene (7.73%), Eugenol (6.33%), 1,8-cineole (5.82 %) and -amorphene (4.76 %) were the principal components comprising the 78.44 % of the essential oil.

According to a study carried out by many authors (Chalchat et al., 1999; Grayer et al., 1996; Marotti et al., 1996; Pascual-Villalobos and Ballesta-Acosta, 2003), linalool and methyl chavicol were the major constituents of O. basilicum oil. The collection studied (see Table 2) comprises seven of the five oil patterns recognized by Grayer et al. (1996) for Ocimum: 1) linalool (OB23), 2) methyl chavicol (OB9, OB10, OB11), 3) linalool and methyl chavicol (OB25, OB34), 4) linalool and eugenol (OB46, OB20) plus additionally a sixth pattern, that of linalool and methyl eugenol-