ORIGINAL ARTICLE

# Antioxidant activity of essential oils obtained from aerial part of some *Lamiaceae* species

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## Abstract

**Introduction:** Essential oils are the perspective source of natural antioxidants because some synthetic antioxidants are now suspected to be potentially harmful to human health. As free radicals can cause inflammatory diseases, cancer, atherosclerosis, and aging, antioxidants can inhibit oxidative chain reactions. Essential oils have a wide range of pharmacological effects as they are multicomponent mixtures of chemical compounds, mainly terpenoids and aromatic components. **Materials and Methods:** The antioxidant activity of essential oils obtained from aerial parts of *Ocimum americanum*, *Lophanthus anisatus, Monarda fistulosa,* and *Satureja hortensis (Lamiaceae)* has been evaluated spectrophotometrically by 2,2-diphenyl-1-picrylhydrazyl free radical scavenging test. **Results:** The assessed samples of essential oils reduced the radical from purple to yellowish color reaching 50% of reduction in the range of concentrations from 2.95 to 9.89 mg/ml. The most prominent antioxidant activity was identified for essential oil of *M. fistulosa* (2.95 mg/ml). The antioxidant activity of four essential oils decreased in such a sequence: *M. fistulosa* > *S. hortensis* > *L. anisatus* > *O. americanum*. **Conclusion:** The results of this study suggest the possibility of using the essential oils of four *Lamiaceae* species as a natural antioxidant sources for the pharmaceutical industry. The antioxidant activities of *S. hortensis* and *M. fistulosa* essential oils with dominated phenolic compounds were the most manifested.

**Key words:** 2,2-diphenyl-1-picrylhydrazyl, antioxidant activity, *Lophanthus anisatus*, *Monarda fistulosa*, *Ocimum americanum*, *Satureja hortensis*, essential oil

## INTRODUCTION

Medicinal plants containing essential oils are an important source to obtain medicines of different pharmacological groups.[1] Essential oils are the perspective source of natural antioxidants because some synthetic antioxidants are now suspected to be potentially harmful to human health.[2-4] As free radicals can cause inflammatory diseases, cancer, atherosclerosis, and aging, antioxidants can inhibit oxidative chain reactions.

Essential oils are an important group of biologically active substances of plants from the *Lamiaceae* Martinov. family.[5-8] Species of this family have a rich usage in phytotherapy due to their curative and preventive properties and also used in cosmetics and food preservation.[9-14] They have a wide range of pharmacological effects as they are multicomponent mixtures of chemical compounds, mainly terpenoids and aromatic components. The essential oils obtained from plants of genus *Ocimum* L., *Lophanthus* L., *Monarda* L., and *Satureja* L. have been studied previously, and their components were identified.[8] It was established the strong antimicrobial activity of essential oils of *Monarda fistulosa* L. and *Satureja hortensis* L. containing aromatic compounds as predominant components. Many scientists associate the antioxidant activity of medicinal products obtained from species of this family with the specific components of essential oils.[2,3,5-7,11,15,16]

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The aim of this study was to determine the antioxidant activity of essential oils isolated from aerial parts of some cultivated in Ukraine *Lamiaceae* species (*Ocimum americanum* L., *Lophanthus anisatus* (Nutt.) Benth., *M. fistulosa,* and *S. hortensis*).

## MATERIALS AND METHODS

### Plant Material

The aerial parts of investigated species were harvested at the flowering stage from the experimental plots in Ternopil region, Ukraine. Herbs were dried at a temperature of 30–35°C in shadow. A voucher specimen of each plant is deposited at the Department of Pharmacognosy and Medical Botany of Ivan Horbachevsky Ternopil State Medical University.

### Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox were purchased from Sigma-Aldrich Chemical Company, Germany. Methanol was from Poch, Poland**.** All the applied reagents and solvents were of analytical grade.

### Sample Preparation

The essential oils were isolated from the powdered herbs (30.0 g) by distillation for 2 h using the Clevenger apparatus. The essential oils were collected from the nozzle of the condenser and dried under anhydrous sodium sulfate and used for the investigation of antioxidant activity by dissolving in methanol in concentrations from 2.5 to 20.0 mg/ml.

### Determination of DPPH Radical Scavenging Activity

The procedure of DPPH radical scavenging activity investigation[17-19] has been adapted for this assay using Hitachi U-2810 UV/VIS spectrophotometer. 0.1 ml of different concentrations of the essential oil in methanol as a sample solution was mixed in the flask with 1.9 ml of DPPH solution obtained by dissolving 2.5 mg DPPH in 100 ml of methanol, and the content was stirred vigorously for 15 s. The DPPH solution was freshly prepared daily and stored in the darkness at room temperature between measurements. Decreasing absorbance of tested mixtures was monitored at 517 nm initially every min (up to 5 min from the beginning of the reaction) and then every 5 min during 30 min after mixing for obtaining kinetics reaction of the process of DPPH reduction. Percentage of DPPH inhibition after 30 min period of reaction was plotted against the essential oil concentration. The value of $IC_{50}$ was calculated. This value means the concentration of a substance required to reduce the radical by 50% ($IC_{50}$).

The mixture consisted of 0.1 ml of the appropriate sample and 1.9 ml of methanol was used as a blank sample. The solution containing 0.1 ml of methanol and 1.9 ml of DPPH solution was used for the determination of the absorbance of DPPH. Trolox was used as a standard antioxidant (positive control),[5,18] and its calibration curve was determined in the range of concentration from 0.01 to 0.1 mg/ml.

The percentage of DPPH free radical-scavenging activity was calculated using the following equation: Percentage inhibition = $[(A_B - A_A)/A_B] \times 100$, where $A_B$ - the absorbance of DPPH and $A_A$ - the absorbance of a test sample. All the experiments were carried out in triplicate.

## RESULTS AND DISCUSSION

The ability of the investigated essential oils to donate a hydrogen atom or electron and scavenge DPPH free radical was determined by the slightly modified method of Brand-Williams *et al*.[18] and Hudz *et al*.[19] In this model, free radicals are reduced and, respectively, decolorized by compounds with antioxidant activity.

The results of the kinetics reaction of Trolox and essential oil DPPH scavenging activity for 30 min are shown in Figures 1 and 2. It is obviously that the decolorizing activity of Trolox as a synthetic antioxidant is manifested already during the 1st min after the start of the reaction [Figure 1]. The essential oils of the investigated plants as shown in Figure 2 on the example of *S. hortensis* exhibit the DPPH scavenging effect much slower compared with Trolox [Figure 1]. Similar differences in properties of synthetic and natural antioxidants have been established by Brand-Williams *et al*.[18]

Scientists[17,19,20] discuss the optimal measurement time of the absorbance for DPPH method because some plant compounds react progressively with reaching a steady state during 2–6 h. The measurement of absorbance using the *Ocimum basilicum* essential oil was carried after 90 min from the beginning of the reaction.[20] Described features of the time interval choices for measuring antioxidant activity create difficulties in comparing the obtained results. At the same time, our research [Figure 2] indicates the possibility

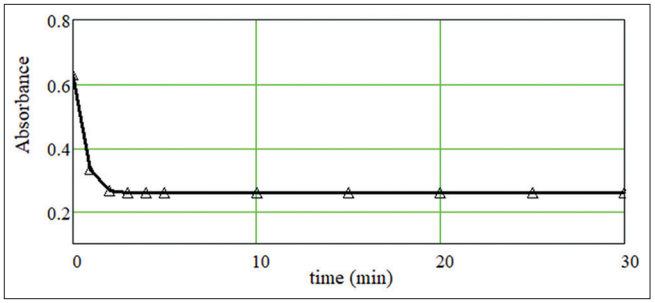


**Figure 1:** The kinetics reaction of 2,2-diphenyl-1-picrylhydrazyl scavenging activity with Trolox (in concentration 0.075 mg/ml)

of these measurements at 30 min after starting of the reaction when the decrease in the absorbance is practically complete.

Study of antioxidant activity of the investigated essential oils and Trolox showed dose-dependent free radical scavenging activity against DPPH [Figures 3 and 4]. As it is shown in Figure 4, $IC_{50}$ of essential oils decreased in the following sequence: *O. americanum* (9.89 mg/ml) > *L. anisatus* (6.38 mg/ml) > *S. hortensis* (4.4 mg/ml) > *M. fistulosa* (2.95 mg/ml). These data show that the best free radical scavenging effect was obtained for the essential oil of *M. fistulosa* which exhibits antioxidant activity at the lowest concentration. The value of $IC_{50}$ for the Trolox was 0.056 mg/ml. The obtained coefficient (0.9987) revealed a very high correlation between the percentage of DPPH inhibition and concentration of Trolox.

It is known from the literature[2,6,15] that antioxidant activity determined by the use of the DPPH method was related to the chemical composition of essential oils as multicomponent mixtures of volatile compounds. As it was previously reported,[8] the major constituents of essential oils obtained from the investigated plants were monoterpenoids and aromatic compounds.

The essential oils of *M. fistulosa* and *S. hortensis* containing such dominating aromatic compounds as thymol and its isomer carvacrol [Figure 5] showed the strongest antioxidant effect in this study. Studies of the qualitative and quantitative composition of oils obtained from these plants[8] revealed the presence of considerable concentrations of phenolic compounds which could be closely related to the high free radical scavenging potential. This potentially correlates with the presence of an electron-donating ortho OH-groups as crucial for exerting an effective antioxidant activity.[7]

The main component of *L. anisatus* essential oil[8] which has been shown the moderate antioxidant properties in this study [Figure 4] is phenylpropanoid methyl chavicol [Figure 5]. Essential oil of *O. americanum* in which monoterpenoid linalool is predominant component revealed the weakest antioxidant property. Similar results were obtained in the investigation with the antioxidant activity of *Hyssopus officinalis* and *O. americanum* essential oils[5,20] which were dominated the monoterpenoids. However, it was established in the investigations *in vivo* that monoterpenoids are generally more lipophilic in the comparison to most aromatic compounds. Therefore, they could play an important role in the protection of cell membrane lipids.[21]

It was considered that many constituents of an essential oils besides the dominant components may contribute to the antioxidant activity, and it is due to the synergy of components' action.[7] It was proved that antioxidant effect of thymol or carvacrol individually is weaker in comparison with a crude essential oil of plants in which they dominated.

As it is known, the component composition of essential oils is affected by climatic factors, growth conditions, and their genetic chemotypes.[22,23] In this regard, the chemical composition of the essential oils of plants of one species, and, consequently,

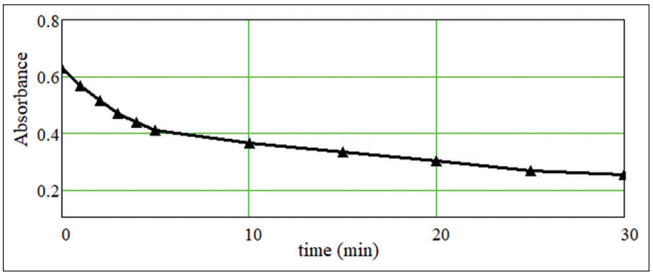


**Figure 2:** The kinetic reaction of 2,2-diphenyl-1-picrylhydrazyl scavenging activity by *Satureja hortensis* essential oils (in concentration 5 mg/ml)

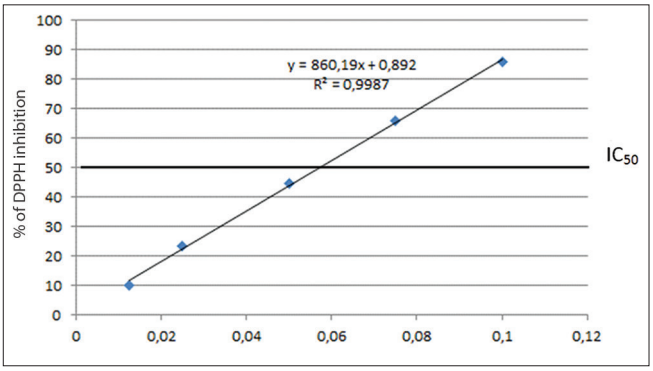


**Figure 3:** $IC_{50}$ values of Trolox after 30 min period of the reaction

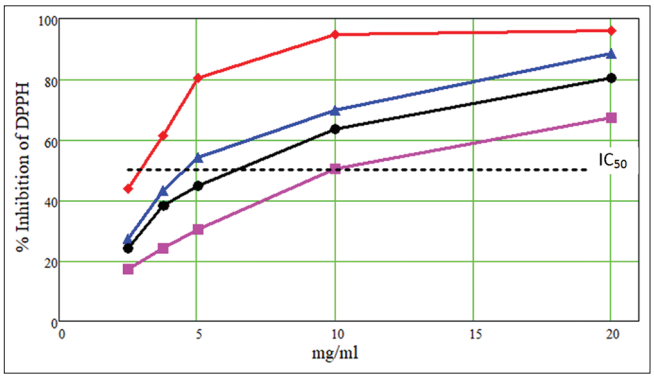


**Figure 4:** $IC_{50}$ values of four essential oils after 30 min period of the reaction: ■ - *Ocimum americanum*; ● - *Lophanthus anisatus*; ▲ - *Satureja hortensis;* ◆ - *Monarda fistulosa*

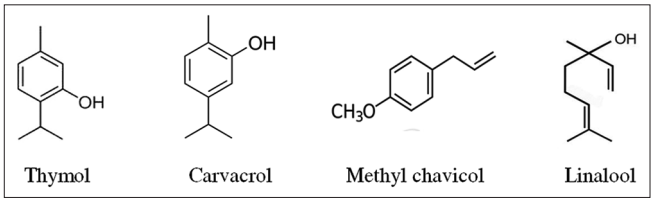


**Figure 5:** Chemical structures of main components of investigated essential oils

their antioxidant activity can differ significantly enough. Thus, the dominant component of the essential oil of *S. hortensis* harvested in Iran was γ-terpinen (31.47%),[23] whereas in the essential oil of this plant cultivated in the western Ukraine the main component was carvacrol (76.16%).[24]

## CONCLUSION

The antioxidant activity of the *O. americanum, L. anisatus*, *S. hortensis,* and *M. fistulosa* essential oils has been evaluated by DPPH radical scavenging test. The tested samples reduced the stable DPPH radical and, respectively, changed purple color of DPPH solutions into the pale yellow color reaching 50% of reduction ($IC_{50}$ values) in the concentration range from 2.95 to 9.89 mg/ml. The antioxidant activity of *S. hortensis* and *M. fistulosa* essential oils with dominating of phenolic compounds was the most manifested. Although the tested essential oils have significant differences in their chemical compositions, all the samples showed effective antioxidant activity which decreased in a following sequence: *M. fistulosa* > *S. hortensis* > *L. anisatus* > *O. americanum.*

The results of this study suggest the possibility of using the essential oils of these four *Lamiaceae* species as a natural antioxidant sources for the pharmaceutical industry. Our results suggest that the essential oils of those species may warrant further investigation for their potential therapeutic efficacy.

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