

ANTIOXIDANT ATTRIBUTES OF FOUR *LAMIACEAE* ESSENTIAL OILS

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Abstract

The present study was conducted to investigate the antioxidant and radical scavenging activities of essential oils of four *Lamiaceae* plants i.e. *Pogostemon cablin*, *Lavandula angustifolia*, *Melissa officinalis*, and *Salvia officinalis* native to Pakistan. The essential oil contents from the aerial parts of *P. cablin*, *L. angustifolia*, *M. officinalis* and *S. officinalis* were found to be 1.98, 0.58, 0.25 and 0.46%, respectively. The principal chemical constituent established in *P. cablin*, *L. angustifolia*, *M. officinalis*, and *S. officinalis* essential oils, were patchouli alcohol, linalool, citronellal, and 1,8-cineol, respectively. The antioxidant activity was evaluated by scavenging of 2,2-diphenyl-1-picryl hydrazyl radical (DPPH[•]), percent inhibition of linoleic acid oxidation and bleaching β -carotene in linoleic acid system. The essential oils possessed appreciable antioxidant and radical scavenging activities revealing potential for therapeutic applications.

Introduction

Essential oils are considered to be one of the potential sources for the screening of anticancer, antimicrobial, antioxidant, and free radical scavenging agents (Jie *et al.*, 2007; Hussain *et al.*, 2008; Shabbir *et al.*, 2009). It is now broadly accepted that certain classes of plant-based compounds such as dietary fiber, phenolic acids, flavonoids, vitamins, radical scavengers and neuropharmacological agents play preventive role against the incidence of some common diseases like cancer, cardiovascular and neurodegenerative disorders, inflammations as well as the ageing process (Marino *et al.*, 2001; Mata *et al.*, 2007). Such natural compounds are believed to demonstrate anticarcinogenic potential and offer diverse health-promoting effects because of their antioxidant attributes (Iqbal *et al.*, 2005; Siddhuraju and Becker, 2007; Liu *et al.*, 2008).

The family *Lamiaceae* consists of about 252 genera and more than 6700 species (Hedge, 1992). Some of *Lamiaceae* species are frequently used in cooked dishes and are recognized as important preventive factor of many diseases (Chalchat and Ozcan, 2008; Hussain *et al.*, 2008; Baser *et al.*, 2009). Essential oils and extracts of these plants are known to possess antiseptic, anti-inflammatory and antimicrobial activities (Burt 2004; Skocibusic *et al.*, 2006; Bozin *et al.*, 2006). *Pogostemon cablin*, *Lavandula angustifolia*, *Melissa officinalis*, and *Salvia officinalis* are underutilized species of *Lamiaceae*.

Even though some reports on the use of plant species as a natural antioxidants are available (Lu and Foo, 2000; Sylvestre *et al.*, 2005; Mata *et al.*, 2007), no such study on the essential oils of *Lamiaceae* species native to Pakistan has been carried. The present study has evaluated the antioxidant and radical scavenging activities of the *Pogostemon cablin*, *Lavandula angustifolia*, *Melissa officinalis*, and *Salvia officinalis* essential oils native to Pakistan.

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Materials and Methods

Materials: The major equipment used were: GC (model-8700, Perkin-Elmer), GC/MS (6890N, Agilent-Technologies, California, USA), UV-VIS spectrophotometer (U-2001, model 121-0032 Hitachi, Tokyo, Japan) and Clevenger-type hydrodistillation apparatus. 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), linoleic acid, dimethylsulfoxide, homologous series of C₉-C₂₄ *n*-alkanes and various reference chemicals used in this study were obtained from the Sigma Chemical Co. (St Louis, MO, USA). All other chemicals (analytical grade) i.e. ferrous chloride, ammonium thiocyanate, hydrochloric acid, chloroform, ethanol, and methanol used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

Plant materials: The aerial parts of *Pogostemon cablin*, *Lavandula angustifolia*, *Melissa officinalis* and *Salvia officinalis* were collected at full bloom from the Botanical Garden, University of Agriculture, Faisalabad, Pakistan. The plant specimens were further identified and authenticated by Dr. Mansoor Hameed, Taxonomist of the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Isolation of essential oils: The essential oils were obtained by a process of hydrodistillation using a Clevenger-type apparatus as described by Hussain *et al.* (2008). Physico-chemical characterization of the essential oils was performed as described earlier (Hussain *et al.*, 2008).

Antioxidant activity

Spectrophotometric DPPH assay: The antioxidant activity of the *Lamiaceae* essential oils was assessed by measuring their ability to scavenging 2, 2'-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The DPPH assay was performed as described by Mimica-Dukic *et al.*, (2003). The samples (from 10 to 500 µg mL⁻¹) were mixed with 1 mL of 90 µM DPPH solution and made up with 95% methanol, to a final volume of 4 mL. Synthetic antioxidant, BHT was used as a positive control. After 1h incubation period at room temperature, the absorbance was recorded at 515 nm. Scavenging (%) of free radicals was calculated using the following formula:

$$\text{Scavenging (\%)} = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

where, A_{blank} is the absorbance of the control (containing all reagents except the test essential oils/compounds), and A_{sample} is the absorbance of the test essential oils/compounds. IC₅₀ values, which represented the concentration of essential oil that caused 50% scavenging, were calculated from the plot of inhibition percentage against concentration.

Percent inhibition in linoleic acid system: The assessment of antioxidant activity of essential oils was also made in terms of percent inhibition of peroxidation in linoleic acid system following a reported method (Iqbal *et al.*, 2005) with slight modification. Essential oils (5 mg) were mixed with a solution of linoleic acid (0.13 ml). Then added 10 mL of ethanol (99.8%) and 10 mL sodium phosphate buffer (0.2 M, pH 7). Total mixture was diluted to 25 ml with distilled water. The solution was incubated at 40°C for 175 h. The degree of oxidation was measured by peroxide value colorimetrically. To 0.2 mL of above sample solution, 10 ml of 75% ethanol was added. This solution then mixed

with 0.2 ml of ferrous chloride solution (20 mM in 3.5% HCl) and 0.2 ml of an aqueous solution of ammonium thiocyanate (30%). The mixture was stirred for 3 min and then absorbance recorded at 500nm. A control was performed with linoleic acid but without essential oils. Synthetic antioxidant, BHT was used as a positive control. Percent inhibition of linoleic acid oxidation was calculated using the following equation:

$$\text{Percent inhibition of linoleic acid oxidation} = \frac{100 - \text{Abs. increase of sample at 175h}}{\text{Abs. increase of control at 175h}} \times 100$$

β -carotene-linoleic acid assay: The antioxidant activity of the essential oils from *Lamiaceae* species was also assessed by measuring the inhibition of the conjugated diene hydroperoxides arising from the linoleic acid oxidation as described by Kulisic *et al.* (2004). A stock solution of β -carotene-linoleic acid mixture was prepared by mixing 25 μ l linoleic acid, 0.5 mg of β -carotene with 1.0 mL of chloroform (HPLC grade) and 200 mg of Tween 40. The chloroform was completely evaporated using a vacuum evaporator at 50°C. Then, 100 ml of distilled water, saturated with oxygen (30 min 100 ml min⁻¹) were added with vigorous shaking. 250 μ l of this reaction mixture was dispensed to test tubes and 350 μ l of the essential oils, prepared at 4 mg mL⁻¹ concentrations were added and the absorbance was immediately (t = 0) measured at 490 nm against a blank, consisting of an emulsion without β -carotene. Then emulsion was incubated for 50 h at room temperature and the absorbance was recorded after this incubation period. The same procedure was repeated with BHT and blank. Antioxidant capacities of the essential oils were compared with BHT and blank.

Statistical analysis: All the experiments were conducted in triplicate and the data are presented as mean values \pm standard deviation of triplicate determinations. Analysis of Variance (ANOVA) was applied using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Ok, USA) software and a probability value of $p \leq 0.05$ was considered to denote a statistical significance difference.

Results and Discussion

The essential oils yields ranged from 0.25 to 1.98% (w/w) based on the dry weight of the plant material (Table 1). The maximum oil contents were found in the *P. cablin* (1.98%), while minimum in *M. officinalis* (0.25%). The variations in the essential oil contents with respect to *Lamiaceae* species were significant ($p < 0.05$). The main components in the essential oil of *P. cablin* as established by GC-MS analysis were patchouli alcohol, α -bulnesene, α -guaiene, γ -patchoulene, β -patchoulene while, the major constituents of *L. angustifolia* essential oil were linalool, linalyl acetate, alloaromadendrene. Citronellal, geraniol, β -citronellol, geranyl acetate, geranial were identified as a main components in *M. officinalis* essential oil. Whereas, *S. officinalis* essential oil mainly contained 1,8-cineol, α -thujone, β -thujone, borneol, camphor.

It was noted that the concentration of major components of the tested essential oils were in partial agreements with the previous reports (Mimica-Dukic *et al.*, 2004; Maksimovic *et al.*, 2007; Hayouni *et al.*, 2008; Wang *et al.*, 2008). The differences in the chemical profiles across countries might have been derived from local, climatic and seasonal factors.

Table 1. Physico-chemical characterization of selected *Lamiaceae* essential oils.

Latin name	Common name	Essential oil yield (g/100g)	Refractive index (25°C)	Density (g cm ⁻³) (25°C)	Major components identified
<i>Pogostemon cablin</i>	Patchouli	1.98 ± 0.09	1.5050 ± 0.030	0.9520 ± 0.010	patchouli alcohol, α -bulnesene, α -guaiene, γ -patchoulene, β -patchoulene
<i>Lavandula angustifolia</i>	Lavender	0.58 ± 0.03	1.4740 ± 0.010	0.890 ± 0.010	linalool, linalyl acetate, allo-aromadendrene
<i>Melissa officinalis</i>	Lemon balm	0.25 ± 0.01	1.4850 ± 0.011	0.895 ± 0.008	citronellal, geraniol, β -citronellol, geranyl acetate, geranial
<i>Salvia officinalis</i>	Salvia	0.46 ± 0.02	1.4620 ± 0.012	0.912 ± 0.011	1,8-cineol, α -thujone, β -thujone, borneol, camphor

Values are mean of three different samples of each *Lamiaceae* species, analyzed individually in triplicate

Table 2. Radical scavenging activity of *Lamiaceae* essential oils in term of IC₅₀ values (μ g/mL)^a.

Essential oils	Radical scavenging activity
<i>Pogostemon cablin</i>	225.7 ± 6.7 ^c
<i>Lavandula angustifolia</i>	289.0 ± 8.5 ^d
<i>Melissa officinalis</i>	69.9 ± 1.9 ^b
<i>Salvia officinalis</i>	62.3 ± 1.8 ^b
BHT	9.9 ± 0.2 ^a

^aValues are mean of three different samples of each *Lamiaceae* species, analyzed individually in triplicate. Mean followed by different letters in superscript represent significant difference among essential oils.

The antioxidant activity of essential oils was assessed by different *in vitro* tests. Free radical scavenging activities of the essential oils were measured by DPPH assay. Free radical scavenging capacity of the tested essential oils increased in a concentration dependent manner. The values for 50% inhibition (IC₅₀) are given in Table 2. According to the results obtained, the essential oils from *S. officinalis* and *M. officinalis* showed excellent radical scavenging activity with IC₅₀ of 62.3 and 69.9 μ g/mL, respectively. The antioxidant activities of *P. cablin* and *L. angustifolia* essential oil were significantly lower than other essential oils and BHT. The variations in the radical scavenging activities of *Lamiaceae* essential oils investigated with respect to species were statistically significant ($p < 0.05$).

To the best of our knowledge, there are very few reports available regarding the DPPH radical scavenging activity of *P. cablin* and *L. angustifolia* essential oils (Mimica-Dukic *et al.*, 2003; Mimica-Dukic *et al.*, 2004; Bozin *et al.*, 2006). According to Mimica-Dukic *et al.*, (2003), the most powerful scavenging compounds were reported to be α - and β -thujone, bornyl acetate, camphor, menthone and 1,8-cineol in the essential oils. The better radical scavenging activity of *S. officinalis* essential oil might be due to the high content of 1,8-cineol.

The antioxidant activity of selected essential oils determined in terms of percent inhibition in β -carotene-linoleic acid system is presented in Figure 1. In general, a similar activity pattern to that seen in the DPPH assay was observed. All the essential oils tested inhibited the oxidation of linoleic acid and that is a big issue in food processing and preservation. Among the essential oils, the strongest effect was supplied by the *M. officinalis* and *S. officinalis* essential oils with 66.5 and 65.2%, respectively. *Pogostemon cablin* showed comparatively weaker activity with 47.3% inhibition of peroxidation. Very few reports found in the literature on the percent inhibition of peroxidation by essential oils using the β -carotene-linoleic acid assay (Hussain *et al.*, 2008).

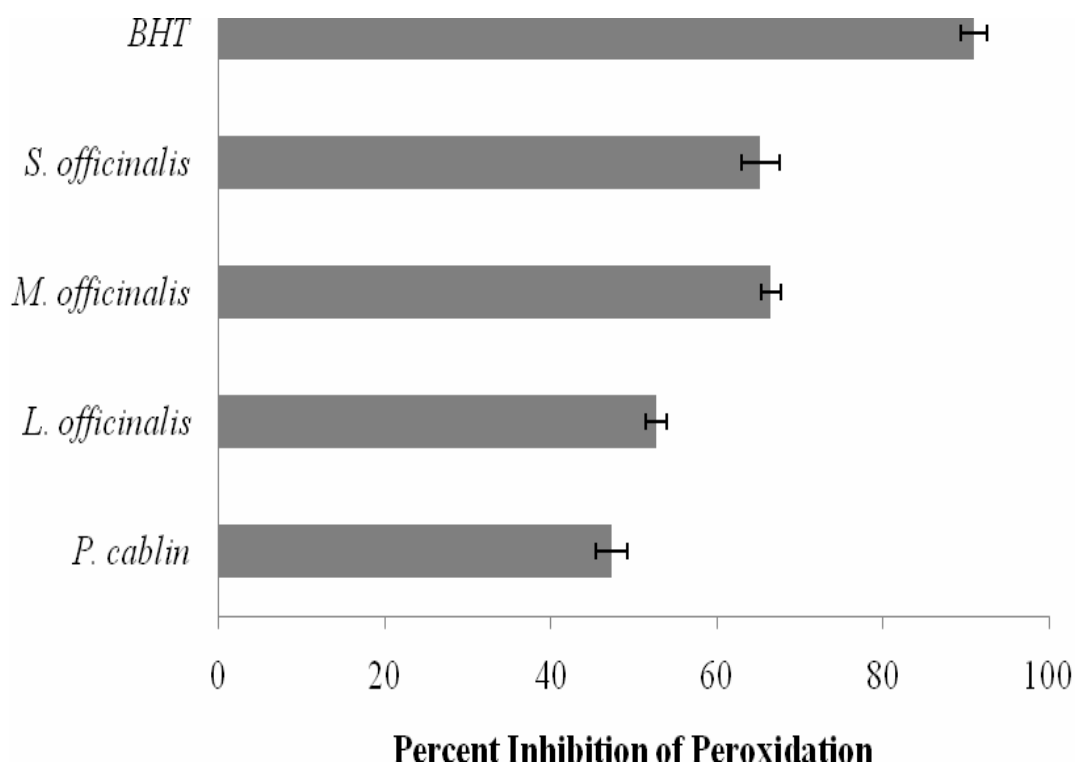


Fig. 1. Antioxidant activity of *Lamiaceae* essential oils in term of inhibition of peroxidation in linoleic acid system.

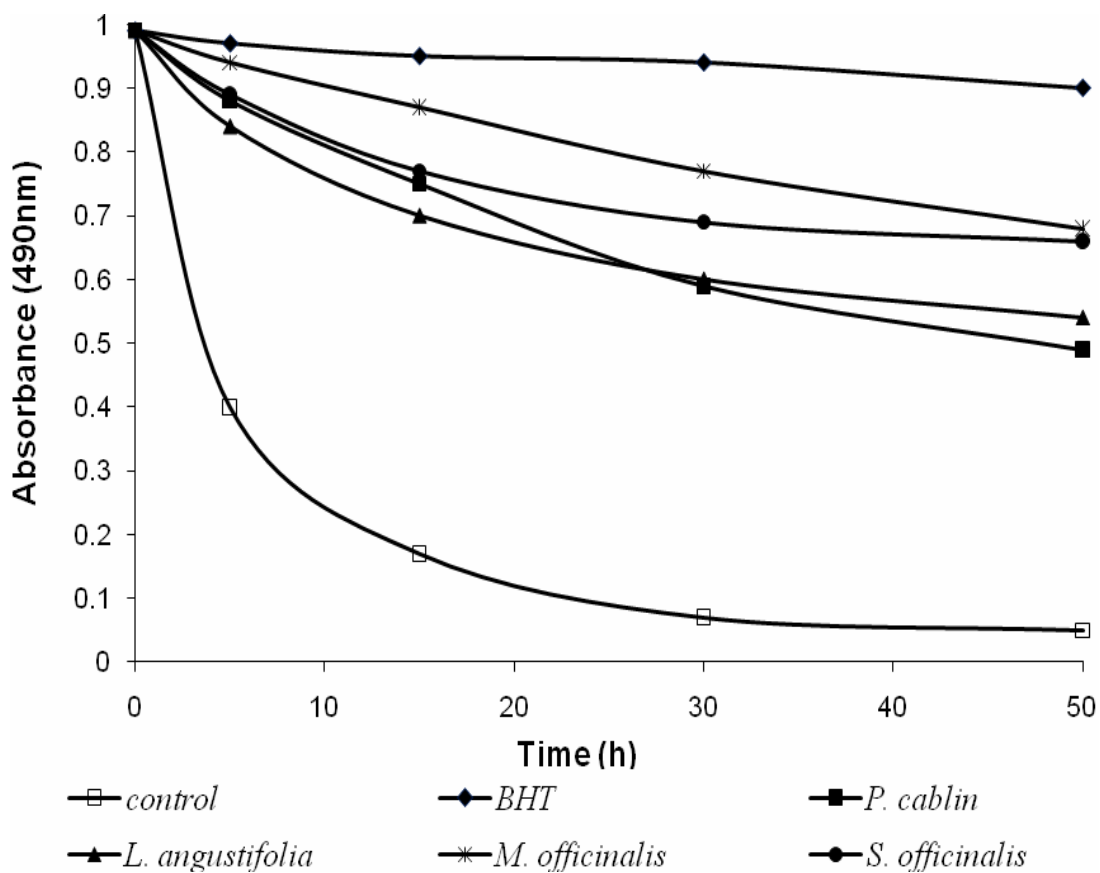


Fig. 2. Antioxidant activity of *Lamiaceae* essential oils measured by bleaching of β -carotene-linoleic acid assay.

Bleaching β -carotene with linoleic acid system as antioxidant activity of *Lamiaceae* essential oils in Figure 2 is presented. The greater the effectiveness of an antioxidant, the slower will be the color depletion. Smaller decrease in absorbance of β -carotene indicates a lower rate of oxidation of linoleic acid and higher antioxidant activity in the presence of essential oils. Control showed the highest rate of color depletion and the least antioxidant activity. Essential oils of *M. officinalis* and *S. officinalis* exhibited better antioxidant activity than essential oils of *L. angustifolia* and *P. cablin*. Based on these results, order of antioxidant activity of selected *Lamiaceae* essential oils was as follows: BHT > *M. officinalis* > *S. officinalis* > *L. angustifolia* > *P. cablin*. No previous data are available in the literature regarding the antioxidant activity of these essential oils using bleaching of β -carotene-linoleic acid assay with which to compare the results of our present experiment.

Conclusion

The essential oils from the investigated *Lamiaceae* plants were rich in oxygenated terpenes and showed considerable radical scavenging and antioxidant activities revealing their potential for therapeutic uses. However, further research is needed to investigate the bioactivity and toxicity of these essential oils using *in-vivo* trials.

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