# ANTIOXIDANT AND ANTIMYCOBACTERIAL ACTIVITIES OF BASIL (*Ocimum gratissimum*) EXTRACTS OBTAINED BY SFE

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The effects of fertilization and harvesting time on the global yield, antioxidant activity, and antimycobacterial activity for the system basil (*Ocimum gratssimum*) + $CO_2$ was studied. The SFE extracts were obtained at 40°C and pressures of 100, 150, 200, 250, and 300 bar. The crude extracts were analyzed by Gas-chromatography/Mass-Spectrometry (GC-MS), and Gas-chromatography/Flame Ionization Detector (GC-FID). The antioxidant activity was assessed using the couple reaction of beta-carotene and linolenic acid, the antimycobacterial activity against *M. tuberculosis* was measured by the Microplate Alamar Blue Assay (MABA) technique. The global yields varied with the fertilization and harvesting period, and were pressure dependent: at 200 bar the highest yield (1.6%) was obtained from plants cultivated with no fertilization; for plants cultivated with fertilization the global yield increase continuously with pressure from 0.6 to 2% (100 to 300 bar). The major compounds in the extracts were eugenol (61%, area), beta-selinene (10%, area), trans-caryophyllene (3%, area), 1,8 cineol (1.5%, area), and alpha-selinene (3%, area). The antioxidant activities were above 90% for all extracts. The lowest MIC (64μg/mL) was detected for the extract obtained at 250 bar.

Keywords: SFE, *Ocimum gratissimum*, basil, antioxidant activity, antimycobacterial activity

## INTRODUCTION

Basil (*Ocimum gratissimum* L.) is an erect small shrub, with height of about 1 m that belongs to the Labiatae family. It is native from India to West Africa but has become naturalized in Europe and America (Iwu, cited by Offiah et al [1]; Paton, cited, by Pino et al, [2]). Even though some work has been done on members of the same genus, little information was reported in the literature about the medical use of basil which its antifungal property, antimutagenic activity, antimicrobial property, immunemodulating effect, smooth muscle contraction effects of lipid-soluble principles and antidiarrheal effects in experimental animals [1]. The Offiah et al [1] investigation revealed that the aqueous extract of basil leaves contains pharmacologically active substance(s), such as antidiarrheal property. This may explain the reason behind the extensive and effective use of the plant as an antidiarrheic agent

in traditional medicine. Recently, Aguiyi et al [3] claimed to be useful in the treatment of diabetes: the hypoglycemic effect of the methanolic extract of basil leaves was evaluated in normal and alloxan-induced diabetes in rats. Essential oils of various *Ocimum* species were studied [4] and verified that basil produces eugenol that is the major constituent in this specie/variety, nonetheless, Silva [5] claimed that there are two chemical types of *Ocimum gratissimum* L.: the thymol and eugenol chemotypes.

## MATERIALS AND METHODS

### Raw material characterization and preparation

Basil was cultivated in the Experimental Farm of Lageado (Plant Production Department, Agronomy Science College/UNESP, Botucatu, SP, Brazil). The plants were cultivated using 0, 4, 8, and 12 $kg/m^2$ of organic fertilizer, using a randomized block design with 4 replications; the harvesting periods were August/2000 (winter), November/2000 (spring), February/2001 (summer), and May/2001 (autumn); the plants were cut at 0.3 m from the ground. The leaves were dried at 37°C.

The dried leaves of basil were comminuted using a mill (Tecnal, model TE-631, series 01071, Piracicaba, Brazil) for 7 s at 14000 rpm; classified according to their sizes using an agitator (Granutest, Abrosinox, Santo Amaro, Brazil) containing standard sieves of the Tyler series for 20 min.; the bed was formed using 40% of particles of mesh 24, 35% of particles of mesh 32, and 25% of particles of mesh 48.

### Basil extracts

The total amount of soluble material (Xo) at given temperature and pressure was determined using a Spe-ed SFE system (Applied Separations, Inc., model 7071, Allentown, USA) equipped with a 3 or 5 mL extraction cell (Thar Designs, Pittsburgh, USA). The bed density was kept at 117.4 kg of basil per cubic meter of bed. The $CO_2$ was admitted into the system at flow rate of $7\times10^{-5}$ kg/s, up to the point where no solute was observed at the exit of the column (approximately 60 min). The amount of the $CO_2$-soluble material was calculated as the ratio of the total mass of extract to the total initial dry mass of basil. The experiments were run at 40°C and pressures of 100, 150, 200, 250 and 300 bar; the assays were duplicated. Carbon dioxide of 99.8% (White Martins Gases Industriais, Campinas, Brazil) was used.

### Characterization of SFE extracts

The basil extracts were analyzed in a GC–MS system (Shimadzu, QP- 5000, Kyoto, Japan), equipped with a fused silica capillary column DB-5 (30 m × 0.25 mm × 0.25 μm, J & W Scientific, Folsom, USA). The electron impact technique (70 eV) was used; the range of masses was 40 to 550 Daltons. The carrier gas was helium (1.7 mL/min.; 99.99% purity, White Martins Gases Industriais, Campinas, Brazil); a sample split ratio of 1/30 was used. The temperatures of the injector and detector were 240 and 230 ºC, respectively. The column was heated to 50 ºC for 5 min., programmed at 4 ºC/min. to 180 ºC and to 280 ºC 15 ºC/min. and kept at 180 ºC for 20 min. One microliter of the samples was injected (5 μL of extract diluted in 1 mL ethyl acetate of chromatographic grade, EM Science, lot 3903991, USA). The identification of the substances was based on (i) comparison of substance mass spectrum with GC–MS system data bank (Nist 62 Library); (ii) comparison of mass spectra with data in literature [6], and (iii) retention index [7]. The quantification of the compounds was done using CG–FID (Shimadzu, model 17A, Kyoto, Japan) operating at the same conditions of the GC–MS.

**Antioxidant activity: Coupled oxidation of linolenic acid and** b**-carotene**

The methodology of Hammerschmidt and Pratt [8] was used with the required modifications for the SFE extracts. The reaction substrate was prepared using 10 mg of β-carotene (99%, Acros, lot B0070834, Pittsburg, USA), 10 ml of chloroform (99,0% PA, Ecibra, lot 13017, Santo Amaro, Brazil), 60 mg of linolenic acid (99%, Sigma, lot U-59A-D4-G, St. Louis, USA) and 200 mg of Tween 80 (Synth, P.A., Diadema, Brazil) This solution was concentrated in a rotary-evaporator (Büchi, model CH-9230, Flawil, Germany or Laborota, model 4001, Viertrieb, Germany) at 50 °C and afterwards diluted with 50 ml of bi-distilled water. The reaction was conducted using the following procedure: to 1 mL of substrate was added 2 mL of bi-distilled water and 0.05 ml of the ginger extract diluted in ethanol (Merck, 99.8% PA, Merck, lot 1216046030, Rio de Janeiro, Brazil) (0.02 g of extract / 1 mL of ethanol). The mixture was set into a water bath (Tecnal, model TE 159, Piracicaba, Brazil) at 40 °C and the reaction product was monitored using a spectrophotometer (HITACHI, model U-3010, Tokyo, Japan) for 0, 1, 2 and 3 hours by taking absorbance readings at 470 nm.

**Antimycobacterial activity**

The antimycobacterial activity was determined for the SFE extracts showed highest antioxidant potency. The minimum inhibitory concentration of the extracts was measured in a Middlebrook 7H9 medium inoculated with *M. tuberculosis* $H_{37}Rv$ - ATCC 27294, *M. tuberculosis* $H_{37}Ra$ - ATCC 25177 using the Microplate Alamar Blue Assay (MABA) technique [9].

**RESULTS AND DISCUSSIONS**

The humidity of the dried leaves was 6.84±0.04 as determined by the infrared method. The global yields obtained at 40$^{o}$C and 150 bar, for the various samples of basil, are in Figure 1. Crops grown at 4 and 12 kg/m$^2$ had a similar behavior. The largest global yield was obtained for the control crop (0 kg/m$^2$ of fertilizer) harvested in the summer. The lowest global yield was determined for the crop grown at 8 kg/m$^2$ and harvested in the summer, in spite of that, the global yield crop increased for the sample harvested in autumn.

It is well documented in literature that the chemical composition of SFE extracts can vary with pressure and temperature; in addition, it can also vary as a result of the retrograde phenomenon. In order to assess the interaction among pressure and harvesting season the global yields isotherms were determined for samples from crops harvested in the summer and grown at 0 and 12 kg/m$^2$ (Figure 2).

Figure 2 shows that the global yield was affected by pressure and the dosage of organic fertilization, also, the effects showed interaction. For the crop grown at 12 kg/m$^2$ the global yield isotherm has the expected behavior: the global yield increases from 100 to 250 bar and remains approximately constant up to 300 bar. For the control crop (0 kg/m$^2$) the global yield increased up to 200 bar and, then, decreased. This behavior could be explained by a modification of the composition of the SFE extracts.

Table 1 shows the compounds identified in the SFE extracts. Table 2 reports the composition of the SFE extracts. The content of eugenol steady increased with pressure for the control crop (0 kg/m$^2$) while the opposite is true for the fertilization dosage of 12 kg/m$^2$, although at a higher eugenol content. The other substances detected in the SFE extracts were: The other compounds in the extracts were beta-selinene (10.2 up to 14.1%, area), trans-caryophyllene (2.8 up to 5.3%, area), 1,8 cineol (traces to 2.6%, area) and alpha-selinene (2.9 up to 3.79%, area).

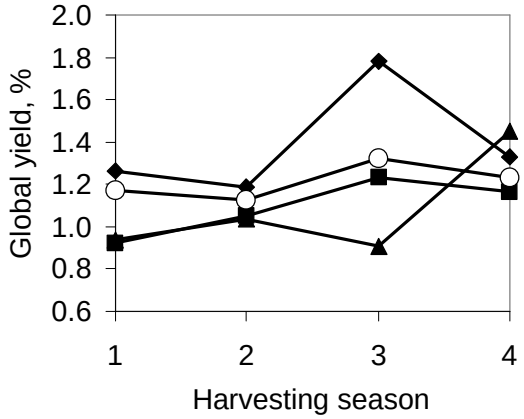


Figure 1: Global yields obtained at 40°C and 150 bar for crops grown at 0 kg/m$^2$ (—◆—), 4 kg/m$^2$ (—■—), 8 kg/m$^2$ (—▲—), 12 kg/m$^2$ (—O—). Harvesting season: 1 (August 2000, winter), 2 (November 2000, spring), 3 (February 2001, summer), 4 (May 2001, autumn).

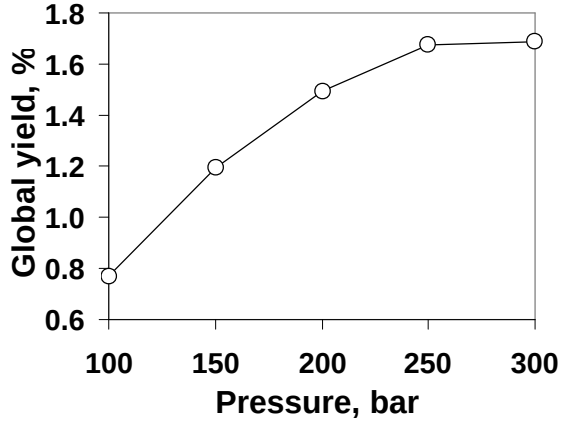


**Figure 2: Global yields as a function of pressure, obtained at 40°C for samples harvested in summer from crops grown at 12 kg/m$^2$ (—O—).**

**Table 1: Compounds identified in the basil extracts for SFE extracts obtained at 150 bar, 40°C, crop grown at 4 kg/m$^2$**

| | Relative proportion, area % | |
|---|---|---|
| | Harvested in the winter (August 2000) | Harvested in the summer (February 2001) |
| Eugenol | 45 | 66.3 |
| 1,8 cineol | 0.5 | 0.9 |
| Trans-caryophyllene | 7.5 | 3.8 |
| Beta- selinene | 28.8 | 16.1 |
| Alpha-selinene | 6.7 | 3.9 |
| Not identified | 12 | 9.0 |

**Table 2: Content of eugenol (relative proportion, area %) present in basil SFE extracts**

| | Eugenol content (relative proportion, area %) | | | | |
|---|---|---|---|---|---|
| | Pressure, bar | | | | |
| Fertilization dosage, kg/m$^2$ | 100 | 150 | 200 | 250 | 300 |
| 0 | 41.5 | 42.7 | 45.0 | 45.2 | 46.6 |
| 12 | 61.0 | 55.4 | 57.2 | 57.9 | 57.7 |

In spite of the differences in the chemical composition of the various samples, their antioxidant activities similar and varied from 97 to 90 % with respect to beta–carotene (Table 3). Table 4 shows the antimycobacterial activities for the various samples. The SFE extracts from the 12 kg/m$^2$ crop had the strongest antimycobacterial activity (lowest minimum inhibition concentration, 64.0 μg/mL).

**Table 3: Antioxidant Activity of *Ocimum gratissimum* L**

| | **Inhibition of oxidation, %** | | | | | |
|---|---|---|---|---|---|---|
| | **Reaction time, h** | | | | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| Control (beta–carotene) | 72 | 57 | 51 | 72 | 57 | 51 |
| Pressure, bar | Fertilizer dosage: 0 kg/m$^2$ | | | Fertilizer dosage: 12 kg/m$^2$ | | |
| 150 | 95 | 91 | 87 | 96 | 93 | 91 |
| 200 | 96 | 93 | 91 | 97 | 93 | 90 |
| 250 | 97 | 95 | 94 | 99 | 97 | 95 |
| 300 | 96 | 93 | 91 | 100 | 99 | 97 |

**Table 4: Minimum Inhibitory concentration (MIC) of SFE extracts against *M. tuberculosis* H37Rv**

| | MIC, μg/mL | |
|---|---|---|
| Pressure, bar | Fertilizer dosage: 0 kg/m$^2$ | Fertilizer dosage: 12 kg/m$^2$ |
| 200 | — | >128.0 |
| 250 | 128.0 | 64.0 |
| 300 | 128.0 | 128.0 |

## CONCLUSIONS

The fertilizer dosage and the harvesting period affected the extraction yield and the chemical composition of the SFE extracts. Nonetheless, their functional properties assessed through the antioxidant and antimycobacterial activities were only marginally influenced.

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