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Functional Properties of Spice Extracts Obtained via Supercritical Fluid Extraction

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In the present study the antioxidant, anticancer, and antimycobacterial activities of extracts from ginger (*Zingiber officinale* Roscoe), rosemary (*Rosmarinus officinalis* L.), and turmeric (*Curcuma longa* L.) were evaluated. The extracts were obtained using supercritical CO_2 with and without ethanol and/or isopropyl alcohol as cosolvent. The extracts' antioxidant power was assessed using the reaction between β-carotene and linolenic acid, the antimycobacterial activity against *M. tuberculosis* was measured by the MABA test, and their anticancer action was tested against nine human cancer ancestries: lung, breast, breast resistant, melanoma, colon, prostate, leukemia, and kidney. The rosemary extracts exhibited the strongest antioxidant and the lowest antimycobacterial activities. Turmeric extracts showed the greatest antimycobacterial activity. Ginger and turmeric extracts showed selective anticancer activities.

KEYWORDS: ***Curcuma longa* L.; *Rosmarinus officinalis* L.; *Zingiber officinale* Roscoe; antioxidant; anticancer activity; antimycobacterial activity**

INTRODUCTION

Extracts of aromatic herbs, spices, and medicinal plants are employed in food processing to impart flavor and other functional properties. The extracts of some of these plants possess antioxidant, bactericidal, and anticancer properties. Although synthetic antioxidants are effective, as is the case of butyl hydroxyanisole (BHA) and dibutyl hydroxytoluene (BHT), there are some restraints to their use because of the evidence that they may be harmful to human health (*1*). For this reason, it is important to consider naturally occurring antioxidants, not only to prevent food degradation but also to formulate functional mixtures for use by the pharmaceutical and cosmetic industries.

Among herbs and spices, ginger (*Zingiber officinale* Roscoe), rosemary (*Rosmarinus officinalis* L.), and turmeric (*Curcuma longa* L.) are known to exhibit antioxidant properties (*1*). Diets that include these natural antioxidants are recognized for reducing the risk of certain chronic diseases such as cancer and cardiovascular disorder (*2*), because, as some researchers suggest, oxidative stress plays an important role in malaria, heart and neurodegenerative diseases, AIDS, cancer, and aging (*3*).

Turmeric is indigenous to South and Southwest Asia and is cultivated in China, the Caribbean Islands, and South American countries (*4*). It belongs to the Zingiberaceae family and is used to impart flavor and yellow color to a number of food formulations (*5*), such as curry. The curcuminoids, found in turmeric, are naturally occurring antioxidants that have a variety of pharmacological properties such as anti-inflammatory and anticancer action (*2*); they have shown anticancer and antimutagenic activities in several animals and cell cultures (*5*). The anticancer activity of turmeric is associated with the presence of the carbonyl group in curcumin (*5*), the most abundant of the curcuminoids of turmeric.

Rosemary (*Rosmarinus officinalis* L.) belongs to the Labiatae family. Rosmanol, the antioxidant present in rosemary, is more effective than both α-tocopherol and BHT (*6*). The volatile oil of rosemary is an antioxidant with a thermal resistance that is stronger than that of BHA and BHT; the latter compounds volatize easily at high temperatures (*7*).

Ginger (*Zingiber officinale* Roscoe) is a branched rhizome that has a characteristic aromatic and pungent flavor (8) belonging to the Zingiberaceae family. It has been cultivated since ancient times for cooking and medicinal preparations. Ginger extracts are rich in gingerols and shogaols, which exhibit

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antioxidant activity (*1*); researchers have demonstrated that other substances present in ginger extracts inhibit the growth of *Mycobacterium avium* and *Mycobacterium tuberculosis* (*9*). Such an observation is important due to the increase in the number of cases of tuberculosis in the past decade, especially in well-developed countries. Also, the increase of multidrug-resistant strains of *M. tuberculosis* to the major chemotherapies used in the conventional therapeutic protocol is responsible for the intensification of research for new drugs; for example, ginger extracts may minimize the problem of immune-depressed individuals, such as HIV-positive patients or patients with chronic diseases (*10*).

Supercritical fluid extraction (SFE) employs solvent above or near its critical temperature and pressure; extraction with pressurized liquids, for example, carbon dioxide at 300 bar (>73.8 bar, the critical pressure) and 30 °C (<31 °C, the critical temperature), is also denoted an SFE process. Because at SFE conditions carbon dioxide is recognized as being an inert solvent, it is the standard choice of solvent for SFE applied to the processing of natural products. For some applications the use of a cosolvent (modifier) is recommended. Because the amount of cosolvent is very small, the advantages of SFE are maintained, despite the required cosolvent removal of the extracts. For instance, for the processing of spices, the use of ethanol or isopropyl alcohol as cosolvent, both classified as GRAS (generally recognized as safe) solvents, will retain all of the SFE advantages.

The objectives of this study were to determine the antioxidant, antimycobacterial, and anticancer activities of SFE extracts obtained with CO_2 and CO_2 plus a cosolvent mixture formed of ethanol and isopropyl alcohol from ginger (*Z. officinale* Roscoe), rosemary (*R. officinalis* L.), and turmeric (*C. longa* L.), and to establish the relationship among the extracts' activities with their composition, which is a function of the operating conditions of pressure, temperature, and percentage of cosolvent.

MATERIALS AND METHODS

Ginger, Rosemary, and Turmeric Extracts. The ginger and turmeric extracts were obtained in previous work at our laboratory (*11*, *12*), whereas the rosemary extracts were prepared for this work. The ginger extracts were stored in a domestic freezer (Brastemp, model 7501, São Paulo, Brazil) at −5 °C for 6 months before they were assayed. The turmeric extracts were assayed just after extraction.

The dried rosemary leaves were purchased from Fazenda Santa Cândida (harvested in August and September of 2000, Campinas, Brazil). The dried leaves were packed in plastic bags, sealed under vacuum (Barbi Industria Mecânica Ltda, São Paulo, Brazil), protected from light to avoid photodegradation, and stored at 20 °C. The rosemary leaves were comminuted in a mill (Brabender OHG, model 981400, series 968052, Duisburg, Germany) and separated according to their sizes (standard testing sieves, series Tyler). Meshes sizes −24 and +48 were selected for the assays. The total amount of soluble material or global yield at a given temperature and pressure was determined using a Speed SFE system (Applied Separations, Allentown, PA) equipped with a 3 or 5 mL extraction cell (Thar Designs, Pittsburgh, PA). The bed density was kept at 119.42 kg of rosemary per cubic meter of bed. The CO_2 was admitted into the system at a flow rate of 7×10^{-5} kg/s, up to the point where no solute was observed at the exit of the column (~60 min). The amount of CO_2-soluble material was calculated as the ratio of the total mass of extract and the total initial mass of rosemary (dry basis). Experiments were run at pressures of 200 and 300 bar and temperatures of 30, 40, and 50 °C; the assays were duplicated. Carbon dioxide 99.8% (White Martins Gases Industriais, 2.8, Campinas, Brazil) or 99.0% (Gama, S.S ONU 1013, Campinas, Brazil) was used.

Turmeric extracts were obtained as described by Braga et al. (*11*) using CO_2 (99.5%, food grade, White Martins Gases Industriais), 6.0−13.8% [wt] of cosolvent, a 1:1 (v/v) mixture of ethanol (99.8%, P.A., Merck), and isopropyl alcohol (99.8%, P.A., Merck) at 30 °C and pressures of 200 and 300 bar. The ginger extracts prepared by Rodrigues et al. (*12*) were obtained at temperatures of 30 and 40 °C and pressures of 100 and 300 bar using CO_2 (99.5%, food grade, White Martins Gases Industriais).

Characterization of the SFE Extracts. The rosemary 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, CA). The electron impact technique (70 eV) was used; the range of masses was 40−550 Da. The carrier gas was helium (1.7 mL/min, 99.99%, White Martins Gases Industriais); a sample split ratio of 1:30 was used. The temperatures of the injector and detector were 240 and 230 °C, respectively. The column was heated to 50 °C for 5 min, programmed at 3 °C/min to 180 °C and to 280 °C at 15 °C/min, and kept at 280 °C for 20 min. One microliter of the samples was injected (0.005 g of extract diluted in 1 mL of ethyl acetate of chromatographic grade, EM Science, lot 3903991, Gibbstown, NJ). The identification of the substances was based on (i) comparison of the substance's mass spectrum with a GC-MS system data bank (NIST 62 Library), (ii) comparison of mass spectra with data in the literature (*13*), and (iii) retention index (*14*). Quantification of the extracts' composition was done using a gas chromatograph (GC-FID Shimadzu, model GC 17A, Kyoto, Japan) under the same conditions described for the GC-MS.

Quantification of the curcuminoids was done using a spectrophotometer (Hitachi, model 3000, UV−visible, Tokyo, Japan); the absorbance was read at 427 nm. The calibration curve was established using curcuminoids of 90% purity (ITAL, Campinas, Brazil) and the following procedure: 0.0053 g of curcuminoids was diluted to 100 mL with ethanol (99.8% P.A. Merck, lot K28659183 104). Aliquots of 2.5 mL (0.25 mL apart) were diluted to 25 mL with ethanol.

Antioxidant Activity: Coupled Oxidation of Linolenic Acid and β-Carotene. The methodology of Hammerschmidt and Pratt (*15*) 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, Pittsburgh, PA), 10 mL of chloroform (99.0% PA, Ecibra, lot 13017, Santo Amaro, Brazil), 60 mg of linolenic acid (99%, Sigma Chemical Co., lot U-59A-D4-G, St. Louis, MO), and 200 mg of Tween 80 (Synth, P.A., Diadema, Brazil). This solution was concentrated in a rotary evaporator (Bucchi, Flawil, Switzerland; or Laborota, model 4001, Viertrieb, Germany) at 50 °C and afterward diluted with 50 mL of bidistilled water. The reaction was conducted using the following procedure: to 1 mL of substrate was added 2 mL of bidistilled water and 0.05 mL of ginger extract diluted in ethanol (99.8% PA, Merck, lot 1216046030, Rio de Janeiro, Brazil) (0.02 g of extract in 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, U-3010, Tokyo, Japan) for 0, 1, 2, and 3 h by taking absorbance readings at 470 nm.

Antimycobacterial Activity. The antimycobacterial activity was determined for the SFE extracts that showed highest antioxidant potency. The minimum inhibitory concentration (MIC) of the extracts was measured in a Middlebrook 7H9 medium inoculated with *M. tuberculosis* H_{37}Rv-ATCC 27294 using the microplate Alamar Blue assay (MABA) (*16*).

Evaluation of the Anticancer Activity. Experiments were performed using the following human cancer cell lines: K562 (leukemia), MCF7 (breast), NCIADR (breast expressing the multidrug resistance phenotype), NCI460 (lung), UACC62 (melanoma), OVCAR (ovary), HT29 (colon), PCO3 (prostate), and 786 (kidney). The National Cancer Institute, Frederick, MD (NCI), kindly donated these cell lines, and stock cultures were kept in liquid nitrogen. Cells were cultured in 25 cm^2 flasks (Nunc Brand Products, Roskilde, Denmark) containing 5 mL of RPMI 1640 (Gibco BRL, Life Technologies, São Paulo, Brazil) with 5% fetal bovine serum (Gibco BRL, Life Technologies). (The cells are used for up to 20 serial passages; afterward, they are discarded and new flasks are unfrozen for use.) All of the adherent cell lines were detached from the culture flasks by the addition of 0.5 mL of trypsin (Nutricell Nutrientes Celulares, Campinas, Brazil). Thereafter, trypsin was inactivated by the addition of 5 mL of 5% serum in RPMI

Table 1. Composition of Turmeric Extract as a Function of Extraction Time Intervals Obtained at 200 bar, 30 °C, 6.0% [wt] Cosolvent (1:1, v/v, Ethanol/Isopropyl Alcohol), and Solvent Flow Rate of 4.09×10^{-5} kg/s

substance	relative propotion, area %, at extraction time (min) interval of 0–75	75–150	150–225	225–350
α-pinene	tr[a]	tr	tr	tr
1,8-cineole	tr	tr	tr	tr
trans-caryophyllene	0.5	tr	tr	tr
ar-curcumene	1.3	1.0	0.8	0.7
α-zingiberene	3.2	2.2	1.6	1.4
β- bisabolene	0.5	tr	tr	tr
β-sesquiphellandrene	2.6	2.0	1.6	1.4
ar-turmerol	0.7	0.8	0.8	0.7
ar-turmerol isomer	0.7	0.9	0.9	0.8
ar-turmerone	15.7	16.2	15.6	15.0
(*Z*)-γ-atlantone	40.1	36.2	32.1	31.8
(*E*)-γ-atlantone	18.2	17.8	16.5	16.2
dihydro-*ar*-turmerone	tr	tr	tr	tr
1-*epi*-cubenol	0.6	0.7	0.6	0.6
6*S*,7*R*-bisabolone	0.8	tr	0.9	0.9
(*Z*)-α-atlantone	0.4	tr	0.7	tr
(*E*)-α-atlantone	tr	tr	tr	0.55
ni[b]	14.8	22.3	27.9	30

[a] tr = area % < 0.4 [b] ni = not identified.

Table 2. Composition of Turmeric Extract as a Function of Extraction Time Intervals Obtained at 300 bar, 30 °C, 6.0% [wt] Cosolvent (1:1, v/v, Ethanol/Isopropyl Alcohol), and Solvent Flow Rate of 3.54×10^{-5} kg/s

substance	relative proportion, area %, at extraction time (min) interval of 0–75	75–150	150–225	225–350
α-pinene	tr[a]	tr	tr	tr
1,8-cineole	tr	tr	tr	tr
trans-caryophyllene	0.4	0.4	0.3	tr
ar-curcumene	1.2	1.1	0.8	0.5
α-zingiberene	3.1	2.7	1.9	1.2
β-bisabolene	0.4	0.5	0.3	tr
β-sesquiphellandrene	2.5	2.4	1.7	1.2
ar-turmerol	0.7	0.8	0.8	0.7
ar-turmerol isomer	0.7	0.8	0.9	0.8
ar-turmerone	15.7	16.0	15.0	13.1
(*Z*)-γ-atlantone	41.3	39.9	35.0	28.7
(*E*)-γ-atlantone	18.6	18.5	16.9	14.4
dihydro-*ar*-turmerone	0.4	0.4	0.4	0.4
1-*epi*-cubenol	0.6	0.6	0.7	0.6
6*S*,7*R*-bisabolone	0.8	0.8	1.0	0.9
(*Z*)-α-atlantone	1.0	0.4	0.4	5.0
(*E*)-α-atlantone	tr	0.4	0.7	1.5
ni[b]	12.6	14.6	23.3	31.1

[a] tr = area % ≤ 0.4. [b] ni = not identified.

1640 medium. Cells were separated into single-cell suspensions by a gentle pipetting action. After counting, the cells were diluted into appropriate seeding densities and inoculated onto 96-wells microtiter plates (Nunc Brand Products). Cell plating volume was 100 μL per well. Seeding densities varied among the cell lines as follows: 6.5×10^4 (K562), 6.5×10^4 (MCF7), 5×10^4 (NCIADR), 4×10^4 (NCI460), and 3×10^4 (UACC62) cells/mL. Microtiter plates containing cells were preincubated for 24 h at 37 °C to allow stabilization prior to the addition (100 μL) of the test substance (crude extract). The plates were incubated with the test substance for 48 h at 37 °C and 5% CO_2. For initial screening, the substances were tested at four concentrations (0.25, 2.5, 25, and 250 μg/mL), and each concentration was studied in triplicate wells. All samples were initially solubilized in dimethyl sulfoxide (DMSO) (Sigma Chemical Co.) at 400 times the desired final maximum test concentration. Extract stocks were stored frozen at −70 °C. The concentrates were then diluted with complete medium containing 50 μg/mL gentamicin (Schering-Plough, Kenilworth, NJ). The sulforodamine B (SRB) assay was performed according to the method of Skehan (*18*). Briefly, the cells were fixed by means of protein precipitation with 50% trichloroacetic acid (TCA) (Sigma Chemical Co.) at 4 °C (50 μL/well, final concentration = 10%) for 1 h. The supernatant was then discarded, and the plates were washed five times with tap water. The cells were stained for 30 min with 0.4% the SRB (Sigma Chemical Co.) dissolved in 1% acetic acid (50 μL/well) (Sigma Chemical Co.) and subsequently washed four times with 1% acetic acid to remove unbound stain. The plates were air-dried, and bound protein stain was solubilized with 150 μL of 10 mM Trizma buffer (Sigma Chemical Co.). The optical density was read on an automated spectrophotometer plate reader at 540 nm. The assays were performed in triplicates.

For cells growing in suspension (e.g., leukemia), the same method was employed, but to fix the cells to the bottom, the TCA concentration was 80%.

RESULTS AND DISCUSSION

The turmeric extracts were characterized with respect to their light (volatile oil) and heavy fractions; the compositions of the volatile oils are shown in **Tables 1** and **2** as a function of the extraction time intervals. The major compounds in the turmeric volatile oil were (*Z*)-γ-atlantone (∼41 to 29%, area), (*E*)-γ-atlantone (∼18 to 14%, area), and *ar*-turmerone (∼16 to 13%, area), regardless of the operating pressure; their contents were higher at the earlier stages of extraction. Except for the relative proportion of (*Z*)-γ-atlantone, the composition of the volatile oil remained approximately constant during the entire extraction process. A similar behavior was observed for the content of curcuminoids: It remained constant up to 150 min and increased from this point to reach a maximum value at 450 min of extraction. In the heavy fraction, the average curcuminoids contents were 0.19% [wt] and 0.21% [wt] at 200 and 300 bar, respectively. No traces of ethanol or isopropyl alcohol were detected in the turmeric extracts; thus, the removal of the cosolvent mixture was effective.

Table 3. Global Yield for the System Rosemary + CO_2 as a Function of Temperature and Pressure

temp, °C	global yield, X_0, (mass of extract/mass of dry rosemary), % 200 bar ρ_{CO_2}, kg/m^3	200 bar X_0	300 bar ρ_{CO_2}, kg/m^3	300 bar X_0
30	890.5	4.1 ± 0.5	948.0	4.5 ± 0.5
40	839.8	3.2 ± 0.5	909.9	4.7 ± 0.5
50	784.3	3.0 ± 0.5	830.4	4.2 ± 0.5

Samples of ginger and turmeric extract were collected at selected time intervals during the extraction process. This provided the opportunity of assessing the effects of the composition of the extracts on their functional properties, for example, antioxidant. Kinetic information related to SFE from ginger can be obtained in Rodrigues et al. (*12*) and from turmeric in Braga et al. (*11*). For these extracts, the functional properties were determined for samples selected from SFE assays that resulted in the largest yields for a given extraction time. Rosemary extracts were obtained through an exhaustive extraction; thus, the term global yield is being used here to indicate that this value is a function of temperature and pressure and is independent of the extraction time. **Table 3** shows the global yields for the system rosemary + CO_2. The global yield was approximately independent of temperature at 300 bar with an average value of 4.4 ± 0.5% [wt]; at 200 bar the global yield

Table 4. Composition of Rosemary Extracts as a Function of the Operating Pressure and Temperature

	relative proportion, area %, at					
	200 bar			300 bar		
	30 °C[a]	40 °C	50 °C	30 °C	40 °C[a]	50 °C
α-pinene	2.1	tr[b]	tr	2.2	tr	tr
1,8 cineole	8.1	4.8	3.9	10.3	7.3	5.6
camphor	33.9	26.8	22.1	39.6	29.2	30.9
borneol	tr	tr	1.9	tr	tr	tr
α-terpineol	3.7	tr	3.1	tr	tr	3.3
verbenone	16.6	18.1	12.8	20.3	15.0	16.2
trans-caryophyllene	11.4	8.6	8.8	10.3	9.8	10.2
nonaeicosane	6.6	9.0	11.7	5.7	9.3	10.4
heneitriacosane	4.9	8.1	9.8	tr	8.3	8.6
ni	12.7	24.6	25.8	11.6	21.1	14.8

[a] CO_2 = 99.8% (White Martins Gases Industriais, 2.8, Campinas, Brazil) was used. [b] tr ≤ 1.4 %. [c] ni = not identified.

decreased from 4.1 ± 0.5% at 30 °C to 3.0 ± 0.5% at 50 °C. The analysis of variance (ANOVA) detected that the effect of pressure over the global yield was significant ($p = 0.009$), whereas that of temperature was not ($p = 0.145$). This behavior, that is, the decrease with temperature at 200 bar, indicates that under the experimental conditions used in this study the system rosemary leaves/carbon dioxide may show retrograde behavior. The behavior of the global yield is analogous to that of the solubility of a solute in a supercritical fluid. There are two main effects of the temperature on solubility (global yield): (i) solute vapor pressure and (ii) solvent density. The CO_2 density decreases as the temperature increases at both pressures, but at 200 bar this effect is the predominant one; consequently, the global yield (solubility) of the rosemary extract decreased and the retrograde phenomenon was observed. At 300 bar, the increase in the solute's vapor pressure with temperature is equally important to the solvent density decrease; thus, the global yield remained constant. **Table 4** shows the composition of the rosemary extracts. The major compound detected in the rosemary extracts was camphor; its relative proportion varied from ~40% (area) at 300 bar/30 °C to ~22% (area) at 200 bar/50 °C. The camphor's relative proportion decreased with temperature at 200 and 300 bar. The verbenone relative proportion varied from ~20% (300 bar/30 °C) to ~13% (200 bar/50 °C). At 200 bar, the relative proportion of *trans*-caryophyllene decreased with temperature, whereas at 300 bar it remained approximately constant; 1,8-cineole, nonaeicosane, and heneitriacosane relative proportions decreased with temperature at both pressures. Only very small amounts of α-pinene, borneol, and α-terpineol were detected.

Generally, the antioxidant activities of vegetable extracts are associated with specific substances. For instance, the antioxidant activity of turmeric extracts is attributed to the presence of the curcuminoids [curcumin, demethoxycurcumin (DMC), and bis-(demethoxycurcumin) (BDMC)] (*2*); the rosemary antioxidant activity is identified with the presence of rosmanol, isorosmanol, carnosol, espirosmaol (*6*), and canosinic acid (*20*). The antioxidant activities of the SFE extracts were superior to the activity of β-carotene (control) (**Table 5**). The antioxidant power of the rosemary extracts remained approximately constant for the 3 h of reaction, whereas for the turmeric and ginger extracts the antioxidant power began at a lower value (22−27%) and increased to values close to that measured for the rosemary extracts (~50%). Thus, rosemary exhibited the strongest antioxidant activity, followed by the ginger and turmeric extracts. The operating conditions (pressure and temperature) have only marginally affected the antioxidant activities of each extract, which can be observed in **Figures 1** and **2**, which show that the antioxidant activity of the turmeric extracts was slightly affected by the extract composition or even by the content of curcuminoids, because the increase in the content of curcuminoids was not followed by an increase in the antioxidant activity. Assays with turmeric extracts obtained using different percentages of the cosolvent mixture showed results similar to that of **Figures 1** and **2**. These results suggest that there is no need to fractionate these vegetable extracts to take advantage of their antioxidant properties.

Rodrigues et al. (*12*) reported small variations in the composition of the ginger extracts as a function of extraction time intervals. Despite this, Zancan et al. (*18*) showed that pressure, temperature, and the use of cosolvents can significantly affect the composition of ginger extracts. These authors demonstrated that α-zingiberene is prevalent in the earlier stage

Table 5. Antioxidant Activity of Rosemary Extracts as a Function of the Operating Temperatures and Pressures

	inhibition of oxidation, %, at		
SFE extract identification	1 h	2 h	3 h
Turmeric (*C. longa* L.)			
200 bar, 30 °C, 6.0% [wt] cosolvent (1:1, v/v, ethanol/isopropyl alcohol, sample collected over extraction time interval of 50–75 min	22	35	41
300 bar, 30 °C, 7.4% [wt] cosolvent (1:1, v/v, ethanol/isopropyl alcohol, sample collected over extraction time interval of 100–125 min	25	38	43
Ginger (*Z. officinale* Roscoe)			
100 bar, 40 °C, 2.5×10^{-5} kg/s, sample collected over extraction time interval of 300–315 min	27	41	44
300 bar, 30 °C, 1.8×10^{-5} kg/s, sample collected over extraction time interval of 345–360 min	27	39	43
Rosemary (*R. officinalis* L.)			
200 bar,[a] 30 °C, 7×10^{-5} kg/s	46	50	53
200 bar, 40 °C, 7×10^{-5} kg/s	44	50	54
200 bar, 50 °C, 7×10^{-5} kg/s	42	47	51
300 bar, 30 °C, 7×10^{-5} kg/s	44	50	53
300 bar,[a] 40 °C, 7×10^{-5} kg/s	42	47	51
300 bar, 50 °C, 7×10^{-5} kg/s	41	45	48

[a] CO_2 = 99.8% (White Martins Gases Industriais, 2.8, Campinas, Brazil) was used.

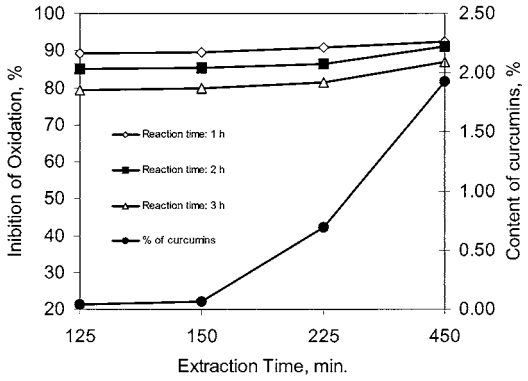

Figure 1. Antioxidant activity as a function of curcumin content of turmeric SFE extracts obtained at 200 bar, 30 °C, and 6.0% [wt] cosolvent (mixture of ethanol/isopropyl alcohol, 1:1, v/v).

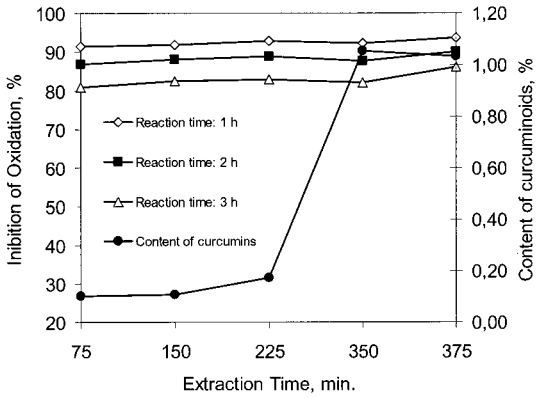

Figure 2. Antioxidant activity as a function of curcumin content of turmeric SFE extracts obtained at 300 bar, 30 °C, and 7.4% [wt] cosolvent (mixture of ethanol/isopropyl alcohol, 1:1, v/v).

Table 6. Minimum Inhibitory concentration (MIC) of SFE Extracts against *M. tuberculosis* H37Rv

SFE extract identification	MIC (μg/mL)
Turmeric (*C. longa* L.)	
300 bar, 30 °C, 3.81×10^{-5} kg/s, 13.8% [wt] cosolvent (1:1, v/v, ethanol/isopropyl alcohol)	15.6
300 bar, 30 °C, 4.04×10^{-5} kg/s, 6.0% [wt] cosolvent (1:1, v/v, ethanol/isopropyl alcohol)	31.25
200 bar, 30 °C, 4.36×10^{-5} kg/s, 6.0% [wt] cosolvent (1:1, v/v, ethanol/isopropyl alcohol), sample collected in the time interval of 50–75 min	31.25
300 bar, 30 °C, 3.54×10^{-5} kg/s, 7.4% [wt] cosolvent (1:1, v/v, ethanol/isopropyl alcohol), time interval of 100-125 min.	31.25
Ginger (*Z. officinale* Roscoe)	
100 bar, 30 °C, 1.75×10^{-5} kg/s, time interval of 330–345 min	31.25
100 bar, 40 °C, 2.5×10^{-5} kg/s, time interval of 300–315 min	31.25
300 bar, 30 °C, 1.8×10^{-5} kg/s, time interval of 345–360 min	31.25
300 bar, 40 °C, 1.72×10^{-5} kg/s, time interval of 240–255 min	31.25
Rosemary (*R. officinalis* L.)	
300 bar,[a] 40 °C, 7×10^{-5}kg/s, extraction time 41 min	128

[a] CO_2 = 99.8% (White Martins Gases Industriais, 2.8, Campinas, Brazil) was used.

of extraction, whereas the content of gingerols and shogaols, the substances associated with antioxidant activities, increased toward the end of the process. Nonetheless, the antioxidant activities reported by Zancan et al. (*18*) were similar to the values obtained in the present study. On the basis of this observation, it may be concluded that the storage time did not affect the antioxidant activity of the ginger extracts.

Table 6 shows the antimycobacterial activities against *M. tuberculosis* (MIC) for the various extracts. The operating conditions used to obtain the ginger extracts did not affect their antimycobacterial activities because their MIC values remained the same for all conditions tested. For turmeric extracts, the use of a large amount of the cosolvent mixture (13.8 % [wt], 1:1, v/v, ethanol/isopropyl alcohol) decreased the value of the MIC from 31.3 to 15.6; the operating temperature and pressure did not affect the MIC. Despite its stronger antioxidant activity, the rosemary extract tested exhibited the lowest antimycobacterial activity (MIC = 128) compared to the ginger and turmeric extracts.

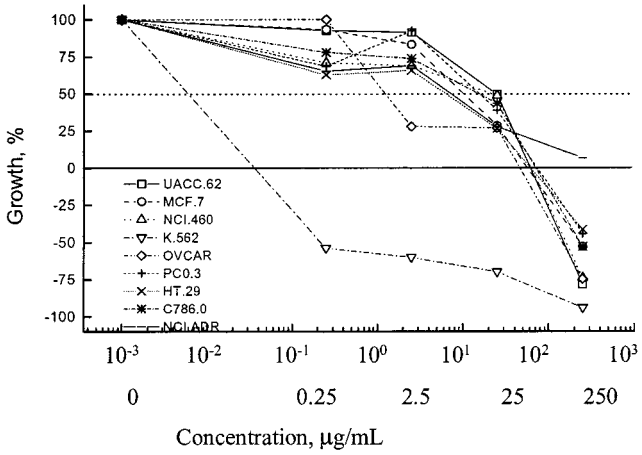

Figure 3. Anticancer activity as a function of cancerous cellular ancestries for turmeric extracts obtained at 200 bar and 30 °C, with ethanol/isopropyl alcohol (1:1, v/v) as cosolvent (6.03%); sample collected over the extraction time interval from 50 to 75 min.

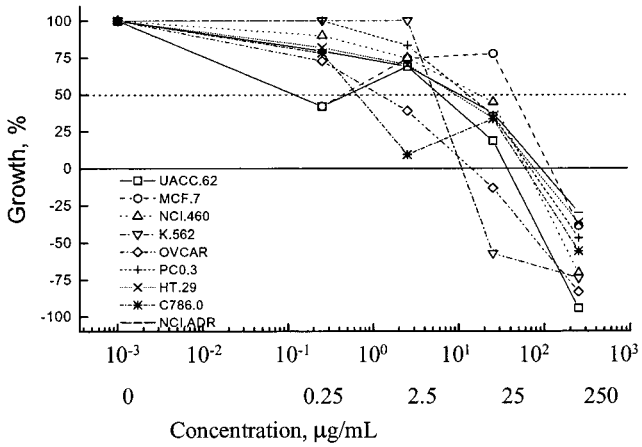

Figure 4. Anticancer activity as a function of cancerous cellular ancestries for turmeric extracts obtained at 300 bar, 30 °C, 13.8% [wt] cosolvent (ethanol/isopropyl alcohol (1:1, v/v), and flow rate of 3.81×10^{-5} kg/s; sample collected over the extraction time interval from 50 to 75 min.

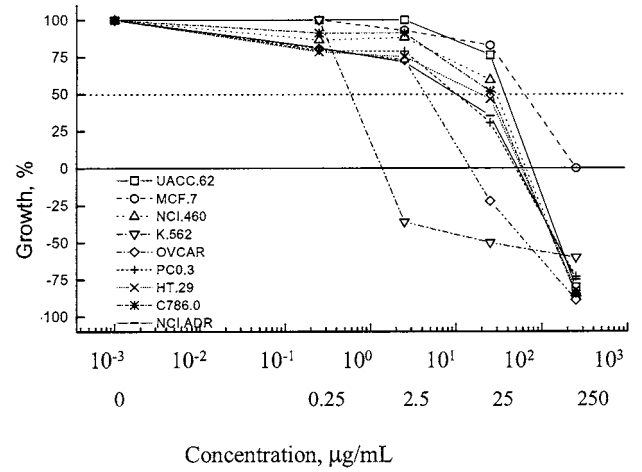

Figure 5. Anticancer activity as a function of cancerous cellular ancestries for ginger extracts obtained at 100 bar and 30 °C; sample collected over the extraction time interval of 120–270 min.

The anticancer activities of ginger and turmeric extracts are presented in **Figures 3**−**5**; because the extracts are considered to be active if their inhibition of growth is >50%, a dashed

line was placed in these figures. As can be observed in **Figure 3**, the turmeric extract, obtained at 200 bar and 30 °C over the time interval from 50 to 75 min, had an anticancer activity that began at 0.25 μg/mL and cytostatic and cytolytic effects, except for NCIADR. These effects were concentration dependent and selective for leukemia (K562). However, because they are ancestor sensitive, such behavior must be reevaluated. For the other cell lines, the cytostatic effect began at 0.25 μg/mL and the cytolytic effect at 250 μg/mL. **Figure 4** shows that the anticancer activity of turmeric extracts obtained at 300 bar and 30 °C (sample collected over the extraction time interval of 100−125 min, 13.8 % [wt] of the cosolvent mixture (1:1, v/v, ethanol/isopropyl alcohol) and a flow rate of 3.81×10^{-5} kg/s) began at 2.5 μg/mL. The cytolytic effect started at 25 μg/mL for OVCAR and NCIADR. **Figure 5** shows the anticancer action of the ginger extract (100 bar, 30 °C, sample collected over the extraction time interval of 330−345 min). The cytostatic and cytolytic effects initiated at 0.25 μg/mL, being concentration dependent and selective for leukemia (K562).

The antioxidant, antimycobacterial, and anticancer activities of the SFE extracts were confirmed. The antioxidant activities of the SFE extracts were superior to the activity of β-carotene, and the rosemary extracts exhibited the strongest antioxidant activity. The lowest values of MIC were detected for turmeric extracts. For certain conditions of extraction the MIC of turmeric and ginger extracts were the same; rosemary's MIC is 4−8 times that of turmeric and ginger. Ginger and turmeric extracts showed selective anticancer activities. Therefore, on the basis of these results, the selection of the spice and the SFE conditions (temperature, pressure, and percentage of cosolvent) will depend on the intended use of the extract.

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LITERATURE CITED

(1) Kikuzari, H.; Nikatani, N. Antioxidants Effects of Some Ginger Constituents. *J. Food Sci.* **1993**, *58*, 1407−1410.

(2) Priyadarsini, K. I. Free Radical Reactions of Curcumin in Membrane Models. *Free Radical Biol. Med.* **1997**, *23*, 838−843.

(3) Aruoma, O. I.; Spencer, J. P. E.; Warren, D.; Jenner, P.; Butler, J.; Halliwell, B. Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem.* **1997**, *60*, 149−156.

(4) Govindarajan, V. S. Turmeric—Chemistry, Technology, and Quality. In *Critical Reviews in Food Science and Nutrition*; Furia, T. C., Ed.; CRC Press: Boca Raton, FL, 1980; Vol. 12, No. 3, p 102.

(5) Chun, K.; Sohn, Y.; Kim, O.; Park, K.; Lee, J.; Moon, A.; Lee, S.; Surh, Y. Anti-tumor promoting potential of naturally occurring diarylheptanoids structurally related to curcumin. *Mutat. Res.* **1999**, *428*, 49−57.

(6) Nakatani, N. Antioxidative Antimicrobial constituents of Herbs and Spices. In *Herbs, Spices and Edible Fungi*; Charalambous, G., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1994; p 764.

(7) Yaakob, B.; Che Man; Irwandi, J. Effects of rosemary and sage extracts on frying performance of refined, bleached and deodorized (RBD) palm olein during deep-fat frying. *Food Chem.* **2000**, *69*, 301−307.

(8) Bartley, J. P. A. New method for the determination of pungent compounds in ginger (*Zingiber officinale*). *J. Agric. Food Chem.* **1995**, *43*, 215−222.

(9) Hiserodt, R. D.; Franzblau, S. G.; Rosen, R. T. Isolation of 6-, 8- and 10-Gingerol from Ginger Rhizome by HPLC and Preliminary Evaluation of Inhibition of *Mycobacterium avium* and *Mycobacterium tuberculosis*. *J. Agric. Food Chem.* **1998**, *46*, 2504−2508.

(10) Dye, C.; Scheele, S.; Dolin, P.; Pathania, V.; Raviglione, M. C. Global Burden of Tuberculosis. Estimated Incidence, Prevalence, and Mortality by Country. *JAMA—J. Am. Med. Assoc.* **1999**, *282*, 677−686.

(11) Braga, M. E. M.; Leal, P. F.; Marques, M. O. M.; Meireles, M. A. A. SFE of Turmeric using CO_2 and Co-Solvents: Selection of Co-Solvent. In *IV Encontro Brasileiro sobre Fluidos Supercríticos (Brazilian Meeting on Supercritical Fluids)—IV EBFS*, Oct 9−11; Salvador, Brazil, 2001; CD-Rom pp 41−47.

(12) Rodrigues, V. M.; Sousa, E. M. B. D.; Monteiro, A. R.; Chiavone-Filho, O.; Marques, M. O. M.; Meireles, M. A. A. Determination of the Solubility of Extracts from Vegetable Raw Material in Pressurized CO_2: a Pseudo-Ternary Mixture Formed by Cellulosic Structure + Solute + Solvent. *J. Supercrit. Fluids* **2002**, *22*, 21−36.

(13) McLaferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*; Wiley: New York, 1989; Vol. 7, p 7872.

(14) Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 1st ed.; Allured Publishing: Carol Stream, IL, 1995; p 468.

(15) Hammerschmidt, P. A.; Pratt, D. E. Phenolic antioxidants of dried soybeans. *J. Food Sci.* **1978**, *43*, 556−559.

(16) Collins, L. A; Franzblau, S. G. Microplate Alamar Blue Assay versus BACTEC 460 System for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.

(17) Skehan, P.; Storeng, R.; Scudiero, D., et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1118.

(18) Zancan, K. C.; Marques, M. O. M.; Petenate, A. J.; Meireles, M. A. A. Extraction of ginger (*Zingiber officinale* Roscoe) oleoresin with CO_2 and co-solvents: a study of the antioxidant action of the extracts. *J. Supercrit. Fluids* **2002**, *24*, 57−76.

(19) Almeida-Dória, R. F.; Regitano-D'Arce, A. F. Antioxidant activity of rosemary and oregano ethanol extracts in soybean oil under thermal oxidation. *Cienc. Tecnol. Aliment.* **2000**, *20*, 197−203 (also available at www.scielo.br).

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