Original

Synergistic and antimicrobial properties of commercial turmeric *(Curcuma longa)* essential oil against pathogenic bacteria

Propriedades antimicrobianas e sinergísticas de óleo essencial comercial de cúrcuma (Curcuma longa) contra bactérias patogênicas

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Abstract

Several studies have shown the antimicrobial and antioxidant properties of turmeric (Curcuma longa), widely used in food industry as a colorant, among other functions. The aim of this study was to determine the antioxidant and antimicrobial properties of turmeric essential oil against pathogenic bacteria and to study the influence of the addition of ascorbic acid on the prevention of polyphenols oxidation. The commercial turmeric essential oil alone did not show bactericidal activity against the microorganisms studied, *Listeria monocytogenes* and *Salmonella typhimurium*, but when combined with ascorbic acid, it showed significant antibacterial activity. The highest antimicrobial activity of turmeric essential oil against *Salmonella typhimurium* was 15.0 ± 1.41 mm at the concentration of 2.30 mg.mL^{-1} of essential oil and 2.0 mg.mL^{-1} of ascorbic acid. With regard to *Listeria monocytogenes*, the largest zone of inhibition (13.7 ± 0.58 mm) was obtained at the same concentrations. The essential oil showed antioxidant activity of EC_{50} = 2094.172 μg.mL^{-1} for the DPPH radical scavenging method and 29% under the concentration of 1.667 mg.mL^{-1} for the β–carotene bleaching method.

Keywords: *turmeric commercial essential oil; ascorbic acid; synergism.*

Resumo

Vários estudos têm demonstrado as propriedades antimicrobianas e antioxidantes da cúrcuma (*Curcuma longa*), a qual é amplamente utilizada na indústria de alimentos, entre outras funções, para colorir. O objetivo deste estudo foi determinar as atividades antioxidantes e antimicrobianas do óleo essencial comercial de cúrcuma contra bactérias patogênicas, além de estudar a influência do ácido ascórbico na prevenção da oxidação dos polifenóis. O óleo essencial comercial de cúrcuma não apresentou atividade bactericida contra os micro-organismos estudados, *Listeria monocytogenes* e *Salmonella typhimurium*, mas, analisado com o ácido ascórbico, apresentou atividade antibacteriana considerável. A maior atividade antimicrobiana do óleo contra *Salmonella typhimurium* foi de 15,0 ± 1,41 mm em uma concentração de 2,30 mg.mL^{-1} de óleo essencial e 2,0 mg.mL^{-1} de ácido ascórbico. Para *Listeria monocytogenes*, o maior halo de inibição (13,7 ± 0,58 mm) foi encontrado nas mesmas concentrações. O óleo essencial apresentou atividade antioxidante de EC_{50} = 2094,172 μg.mL^{-1}, para o método do radical DPPH•, e de 29% na concentração de 1,667 mg.mL^{-1}, pelo método de descoloração do sistema β - caroteno/ácido linoleico.

Palavras-chave: *óleo comercial essencial de cúrcuma; ácido ascórbico; sinergismo.*

1 Introduction

A recent trend in the food industry is the gradual reduction of chemical additives. In order to accomplish this task, industries have been searching for alternative compounds that control the microbial growth and enhance oxidative stability (SOUZA et al., 2005). Turmeric is an herbaceous evergreen plant in the Zingiberaceae family, which can be found in many locations such as China, India, and some countries of South America (SCARTEZZINI; SPERONI, 2000). Several studies have shown the antioxidant and antimicrobial properties of Turmeric essential oils (NEGI et al., 1999; NEGHETINI, 2006; NAZ et al., 2010). In Brazil, turmeric use has increased mainly due to its coloring property and its ability to improve food odor (CHASSAGNEZ; CORRÊA; MEIRELES, 1997; NEGHETINI, 2006). It is mainly found in ready-to-eat soups, ice-creams, and pasta. The most important compounds responsible for the antioxidant activity of turmeric are phenolic compounds, such as curcuminoid dyes and essential oils (BURKE, 1994; NEGHETINI, 2006). Phenolic compounds exhibit the ability to destroy bacterial cell walls and penetrate into microbial cell affecting its metabolism (MARINO; BERSANI; GIUSEPPE, 2001).

Commercial essential oils have low biological activity due to their extraction and handling processes (HENTZ; SANTIN, 2007; BENELLI, 2010). This behavior is the reason of the growing interest for synergistic compounds that increase the antimicrobial activity of these oils. Accordingly, ascorbic acid is known as the most important natural antioxidant compound present in our daily diet. In addition, it exhibits

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many physiological and anticarcinogenic activities (LEE et al., 2002; ZAMBUCHINI et al., 2008).

This study aims to estimate the antimicrobial and antioxidant activities of commercial turmeric (*Curcuma longa*) essential oil and to evaluate the influence of the addition of ascorbic acid on the prevention of polyphenols oxidation.

2 Materials and methods

2.1 Essential oil and physical-chemical analysis

Commercial turmeric essential oil was purchased from an UK industry. The manufacturer description included: plant from India, oil obtained from vapor distillation of the treated rhizome with 100% of purity. The oil was yellow in color and exhibited a strong and characteristic odor.

Specific weight (γ) and Refractive index analysis

The weight of a sample of 1 mL of essential oil was measured at 25 °C (Analytical balance AY220, SHIMADZU do Brasil Ltda., São Paulo/SP, Brazil). The specific weight was expressed as $g.mL^{-1}$ of essential oil, and the refractive index analysis was determined using a refractometer (Abbé WYA, 2-WAJ) at 20 °C.

2.2 Antimicrobial activity

Disk diffusion method

The antimicrobial activity of the essential oil against *Listeria monocytogenes* Scott A and *Salmonella typhimurium* ATCC 12228 was tested. The bacteria were cultivated in BHI (brain heart infusion) broth at 36 °C for 24 hours. Bacteria at the concentration of 10^6 $CFU.mL^{-1}$ were inoculated in Mueller Hinton Agar (Merck S.A., Brazil). After inoculation, sterile filter paper disks of 6 mm diameter were placed on the surface of the agar, and solutions at the concentrations of 0.05; 0.10; 0.30; 0.50; 0.70; 0.90; 1.00; 1.50; 2.00; 2.30 $mg.mL^{-1}$ of essential oil were slowly inserted into the disks with a micropipette of 25 μL. The same amounts of essential oil were tested with the addition of 0.8 $mg.mL^{-1}$ and 2.0 $mg.mL^{-1}$ of L(+) ascorbic acid analytical grade (Dinâmica, Brazil). Pure vitamin C control tests at the above mentioned concentrations were conducted for comparison purposes.

Amoxicillin/clavulanic acid was used as the control sample for *Salmonella typhimurium*; ampicillin for *Listeria monocytogenes*; and gentamicin for both microorganisms.

The experiments were performed in triplicate, and the results were expressed in mm as the arithmetic mean of the inhibition zone values obtained. The data (means) were analyzed by ANOVA and Tukey tests ($p < 0.05$) using the Statistica 7.0 software (Statsoft Inc., USA). Table 1 summarizes the tests used in this study.

2.3 Antioxidant activity

DPPH radical scavenging method

Antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical 2.2 diphenyl-1-picrylhydrazyl (DPPH), as described by Mensor et al. (2001).

The experiment consisted in the incubation of an ethanolic solution of DPPH, 0.3 mM, (Sigma - Aldrich, USA), for 30 minutes, added with essential oil at the concentrations of 5; 10; 25; 50; 125; 250; 500 $\mu.mL^{-1}$ in ethanol analytical grade (Merck S. A., Brazil), followed by an absorbance measurement at 517 nm using a FEMTO UV-Vis spectrophotometer (800XI - São Paulo, Brazil). The procedures were similar to those of the control sample, which consisted of the ethanolic DPPH solution without antioxidant. The blank sample contained solvent ethanol. The inhibition percentage of the sample tested on the DPPH radicals was estimated by Equation 1:

$$AA\% = 100 - \frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \quad (1)$$

where $AA\%$ is the percentage of antioxidant activity, Abs_{sample} is the absorbance of the sample, $Abs_{control}$ is the absorbance of the control sample, and Abs_{blank} is the absorbance of the blank sample.

The concentration necessary to scavenge 50% of the free radical DPPH (EC_{50}) was estimated by linear regression.

β–*carotene bleaching method*

The method is based on measurements of β–carotene bleaching, which is generated by linoleic acid oxidation in an emulsion. The β–carotene loses the yellow color due to its reaction with radicals formed in this oxidation (MATTHÄUS, 2002; KANG et al., 2006). The rate of β–carotene bleaching was evaluated by the difference between the initial absorbance readings at 470 nm and after 120 minutes. The values of the percentage of antioxidant activity (AA %) were estimated by

Table 1. Description of the tests performed for evaluation of antimicrobial activity of commercial turmeric (*Curcuma longa*) essential oil using the disk diffusion method.

Test	Description
A	Commercial turmeric (*Curcuma longa*) essential oil against *Listeria monocytogenes*
B	Commercial turmeric (*Curcuma longa*) essential oil added with 0.8 $mg.mL^{-1}$ of ascorbic acid against *Listeria monocytogenes*
C	Commercial turmeric (*Curcuma longa*) essential oil added with 2.0 $mg.mL^{-1}$ of ascorbic acid against *Listeria monocytogenes*
D	Commercial turmeric (*Curcuma longa*) essential oil against *Salmonella typhimurium*
E	Commercial turmeric (*Curcuma longa*) essential oil added with 0.8 $mg.mL^{-1}$ of ascorbic acid against *Salmonella typhimurium*
F	Commercial turmeric (*Curcuma longa*) essential oil added with 2.0 $mg.mL^{-1}$ of ascorbic acid against *Salmonella typhimurium*

Equation 2. Absorbance analysis was measured using a FEMTO UV-Vis spectrophotometer (800XI - São Paulo, Brazil)

$$AA\% = \left\{1 - \left[\frac{Abs_{t,120} - Abs_{t,0}}{Abs_{controlt,120} - Abs_{controlt,0}}\right]\right\} \times 100 \quad (2)$$

where $AA\%$ is the percentage of antioxidant activity, $Abs_{t,120}$ is the absorbance of the sample after 120 minutes, $Abs_{t,0}$ is the absorbance of the sample at $t = 0$, $Abs_{controlt,120}$ is the absorbance of the control sample after 120 minutes, and $Abs_{controlt,0}$ is the absorbance of the control sample at $t = 0$.

2.4 Analysis of the total content of the phenolic compounds

The analysis of the total content of phenolic compounds was conducted by the Folin-Ciocalteu method (Sigma-Aldrich, USA - SINGLETON; ROSSI JUNIOR, 1965; PESCHEL et al., 2006) with standard curve of galic acid - GAE (Nuclear, CAQ Ind. e Com. Ltda., Brazil). In order to measure the absorbance, a FEMTO UV-Vis spectrophotometer (800XI - São Paulo, Brazil) at 765 nm was used. The results were expressed as milligrams of total phenolic compounds in galic acid per gram of turmeric essential oil (mg GAE.g^{-1}).

3 Results and discussion

3.1 Physicochemical analysis

The specific weight value found was 0.8948 g.mL^{-1}, and the refraction index of the commercial essential oil was 1.4917. Krishnamurthy et al. (1976) and Govindarajan and Stahl (1980) found close results for the specific weight of Indian Turmeric: 0.929 g.mL^{-1} at 20 °C and 0.9423 g.mL^{-1} at 24 °C. In addition, similar results were obtained for the Brazilian Turmeric: 0.9068 g.mL^{-1} at 30 °C and 0.906 g.mL^{-1} at 25 °C (PÉRET-ALMEIDA, 2006; NEGHETINI, 2006). The differences in these results may be attributed to differences in raw material, maturation of the samples, solvent used, and distillation time (GOVINDARAJAN; STAHL, 1980). The refraction index results found in the literature were 1.5069 at 25 °C (NEGHETINI, 2006), 1.5067 at 30 °C (PÉRET-ALMEIDA, 2006), and 1.5054 at 30 °C (GOVINDARAJAN; STAHL, 1980).

3.2 Antimicrobial activity

Method of disk diffusion

The initial tests conducted with the pure commercial sample of the essential oil did not show antimicrobial activity against *Listeria monocytogenes* Scott A and *Salmonella typhimurium* ATCC.

However, after the addition of ascorbic acid, the resulting mixture showed antimicrobial activity against the two above mentioned microorganisms, as shown in Table 2. The results agree with those obtained by Nanasombat and Lohasupthawee with ethanolic extract (2005), Franco et al. with essential oil (2007), and Fagbemi et al. (2009) with turmeric aqueous extract (*Curcuma longa*). Other studies showed that turmeric exhibits antimicrobial activity against fungi and non-pathogenic bacteria (NEGHETINI, 2006; NAZ et al., 2010).

It can be seen that this essential oil at concentrations ranging from 0.05 to 2.30 mg.mL^{-1} added to ascorbic acid at concentrations of 0.8 and 2.0 mg.mL^{-1} (tests B and C), exhibited inhibition zones between 9.0 ± 1.0 and 13.7 ± 0.58 mm against *Listeria monocytogenes*. The maximum inhibition of both bacteria was obtained with 2.30 mg.mL^{-1} of oil added to 2.0 mg.mL^{-1} of ascorbic acid. The commercial oil added to 0.8 mg.mL^{-1} of ascorbic acid presented antimicrobial activity only against *Salmonella typhimurium* at the concentration of 2.0 mg.mL^{-1}. This behavior can be attributed to the larger complexity of the double cell wall of Gram-negative bacteria,

Table 2. Antimicrobial activity of commercial turmeric (*Curcuma longa*) essential oil against pathogenic bacteria with filter paper disk of 6 mm.

Concentration of oil (mg.mL^{-1})	Inhibition zone (mm)[(1)]					
	Test A	Test B	Test C	Test D	Test E	Test F
0.05	nd	9.0 ± 1.0^{a}	9.3 ± 0.6^{a}	nd	nd	10.2 ± 1.0^{a}
0.10	nd	9.0 ± 1.0^{a}	9.7 ± 0.6^{a}	nd	nd	10.7 ± 0.6^{a}
0.30	nd	9.0 ± 1.0^{a}	9.7 ± 0.6^{a}	nd	nd	11.2 ± 0.8^{b}
0.50	nd	9.0 ± 1.7^{a}	10.0 ± 0.0^{a}	nd	nd	11.8 ± 0.3^{b}
0.70	nd	9.7 ± 0.6^{a}	11.2 ± 0.3^{b}	nd	nd	12.2 ± 1.0^{b}
0.90	nd	10.0 ± 0.0^{a}	12.4 ± 0.5^{b}	nd	nd	12.2 ± 1.0^{b}
1.00	nd	10.0 ± 1.0^{a}	12.6 ± 0.8^{b}	nd	nd	12.7 ± 0.3^{b}
1.50	nd	10.0 ± 0.0^{a}	12.6 ± 0.8^{b}	nd	nd	13.7 ± 2.0^{b}
2.00	nd	11.7 ± 1.5^{ab}	13.5 ± 1.3^{ab}	nd	11.3 ± 1.5^{a}	14.8 ± 1.8^{b}
2.30	nd	12.5 ± 0.7^{a}	13.7 ± 0.6^{ab}	nd	12.7 ± 0.6^{a}	15.0 ± 1.4^{b}
Control sample	*Listeria monocytogenes*				*Salmonella typhimurium*	
Vit. C (0.8 mg.mL^{-1})	Nd				nd	
Vit. C (2.0 mg.mL^{-1})	10.2 ± 1.1^{a}				8.13 ± 1.2^{a}	
Ampicilin	18.3 ± 1.7				-	
Amo/Clavulanic acid	-				20.7 ± 0.6	
Gentamicin	22.0 ± 0.0^{a}				23.7 ± 0.6^{b}	

[(1)]Lines and rows means with different letters differed significantly ($p < 0.05$). nd = not detected, - = not tested.

such as *Salmonella typhimurium* (HAMMER; CARSON; RILEY, 1999; CANSIAN et al., 2010).

Pure vitamin C control at 0.8 mg.mL^{-1} did not prevent growth of both bacteria. On the other hand, the use of 2.0 mg.mL^{-1} showed an inhibition zone of 10.2 ± 1.08 mm for *Listeria monocytogenes* and of 8.13 ± 1.21 for *Salmonella typhimurium*. These results indicate a larger resistance of *Salmonella typhimurium* to the pH ranges used in this study. Therefore, it can be observed a synergism between the essential oil and the ascorbic acid.

Many studies showed that the synergism between ascorbic acid and phenolic compounds is due to the ascorbic acid ability to prevent the oxidation of the phenolic compounds. Since phenolic compounds are the main responsible for the antimicrobial activity of the turmeric oil, ascorbic acid favors this activity (NEGHETINI, 2006; HATANO et al., 2008; ZAMBUCHINI et al., 2008; NAZ et al., 2010). Studies on the interaction between turmeric essential oil and vitamin C were not found in the literature.

3.3 Antioxidant activity

DPPH radical scavenging method

This test measures the hydrogen-atom- or electron-donating ability of the essential oil to convert DPPH into the form DPPH – H (diphenylpicrylhydrazyn) (CANSIAN et al., 2010). The experimental results were expressed as the reduction of the free radical DPPH by 50%. The parameter EC_{50}, which represents the inhibitory concentration necessary for this reduction, was used (QIAN; NIHORIMBERE, 2004; ARBOS et al., 2010).

The linear regression of the antioxidant activity (%) and the oil concentration (g.mL^{-1}) ($y = 0.0227x + 2.462, R^2 = 0.862$) provided a value of 2094.2 µg.mL^{-1} to EC_{50}, which has high magnitude when compared to reference standards such as EC_{50} = 2.15 µg.mL^{-1} for ascorbic acid and EC_{50} = 5.37 µg.mL^{-1} for BHT (CANSIAN et al., 2010). Table 3 presents some published results of EC_{50} obtained using the DPPH method for some species of plants and different solvents used to extract essential oils. Only a few studies containing data on antioxidant capacity of commercial essential oils are available in the literature. Benelli (2010) did not report finding antioxidant activity for commercial essential oil of orange for the concentration of 500 µg.mL^{-1}. In the same study, turmeric commercial essential oil exhibited 14% of antioxidant activity.

β–carotene bleaching method

The antioxidant activity percentage (AA%) found for curcuma essential oil using the β–carotene bleaching method was 29% for the concentration of 1.667 mg of essential oil per mL of ethylic alcohol. The antioxidant activity obtained by linear regression for the concentration of 1.667 mg.mL^{-1} using the DPPH radical scavenging method was about 40%. The two methods furnished discordant results for the antioxidant activity, which can be attributed to the high solubility of flavonoids in polar solvents, such as the ethanol used to solubilize the radical DPPH. Aqueous and lipidic phases, which have low solubility, are used in this method instead of polar solvents (RICE-EVANS; NICHOLAS; PAGANGA, 1996; OLDONI, 2007).

Table 4 shows published results of antioxidant activity using for different species of plants using different extraction methods.

3.4 Analysis of the total content of phenolic compounds

The total content of phenolic compounds found was 56.79 ± 1.37 mg GAE.g^{-1}. Chen et al. (2008) found 21.4 ± 1.7 mg GAE.g^{-1} for methanolic extract of *Curcuma longa* L., 33.4 ± 5.7 mg GAE.g^{-1} for *Curcuma zedoaria*, and 35.6 ± 5.5 mg GAE.g^{-1} for *Curcuma domestica*. Herrero et al. (2010) obtained values between 70 and 120 mg GAE.g^{-1} for Rosemary extract using different solvents. This compound is often used as antioxidant in food formulations.

Commercial turmeric (*Curcuma longa*) essential oil was more effective considering its total content of phenolic compounds compared to the published results of methanolic extract, aqueous solution, and essential oil obtained in laboratory. In addition, the results obtained were close to those of the Rosemary essential oil.

Table 3. Effective concentration at 50% (EC_{50}) for different species of plants and different solvents used to extract the essential oil obtained by the DPPH radical scavenging method.

Species		Extraction	EC_{50} µg.mL^{-1}	Source
Common name	Scientific name			
Curcuma-selvagem	*Curcuma aromatica* Salisb.	Essential oil (hydro-distillation)	14.45	Al-Reza et al. (2010)
		Extract (methanol)	16.58	
		Extract (cloroformium)	38.86	
		Extract (hexane)	45.97	
Guaco	*Mikania glomerata* Spreng.		1283.88	Vicentino and Menezes (2007)
Ho-sho	*Cinnamomum camphora* Ness	Essential oil (hydro-distillation)	12942.00	Cansian et al. (2010)
Oregano	*Origanum dictamnus*	Extract (petroleum ether)	8385.00	Kouri et al. (2007)
Orange	*Citrus sinensis* L. Osbeck	Commercial essential oil (cold pressing)	nd	Benelli (2010)

nd = antioxidant activity not detected at concentrations up to 500 µg.mL^{-1}.

Table 4. Results of the antioxidative activity (AA%) obtained using the β–carotene bleaching method for different plant species and different extraction techniques.

Species		Extraction	Concentration ($mg.mL^{-1}$)	AA (%)	Source
Common name	Scientific name				
Turmeric	*Curcuma longa*	Essential oil (hydro-distillation)	40.00	72.40	Sacchetti et al. (2005)
Rosemary	*Rosmarinus officinalis*	Essential oil (hydro-distillation)	40.00	81.10	
Orange	*Citrus sinensis* L. Osbeck	Commercial essential oil (cold pressing)	1.67	17.00	Benelli (2010)
Alecrim-de-angola	*Vitex agnus castus* L.	Essential oil (hydro-distillation)	2.00	86.17	Sarikurkcu et al. (2009)
		Extract (hexane)	2.00	33.75	
		Extract (methanol)	2.00	53.14	
Oregano	*Origanum vulgare* L.	Essential oil (hydro-distillation)	2.00	64.00	Kulisic et al. (2004)

4 Conclusions

The results of the specific weight and refraction index of commercial turmeric (*Curcuma longa*) essential oil indicate that it may contain some impurities due to extraction and handling processes.

Like the turmeric oils and extracts obtained in laboratory, the commercial essential oil did not exhibit antimicrobial activity against *Listeria monocytogenes* Scott A and *Salmonella typhimurium* ATCC. The addition of ascorbic acid to inhibit oxidation of phenolic compounds may be an alternative to increase the antimicrobial activity of the commercial turmeric essential oil. The results of antioxidant activity presented in this study were lower than the results published for both methods employed in this study. Further studies are necessary in order to obtain a better characterization of the antimicrobial and antioxidant properties of commercial Turmeric essential oil and its behavior when added with ascorbic acid.

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