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CHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITY OF BOLDO (*Peumus boldus* MOLINA) EXTRACTS OBTAINED BY COMPRESSED CARBON DIOXIDE EXTRACTION

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Abstract - This work reports the effects of temperature (20 to 50°C) and pressure (100 to 250 bar) on the extraction yield, chemical characteristics and antimicrobial activity of extracts of *Peumus boldus* Molina obtained by compressed carbon dioxide extraction. Results showed that the extraction variables affect the extraction yield and the chemical distribution of the major compounds present in the extracts. The extracts were chemically analyzed with regard to 1,8-cineole, trans-sabinene, pinocarveol, pinocarvone, 4-terpineol, ascaridole, piperitone oxide, limonene dioxide and n-eicosane in a GC/MSD. Antimicrobial tests demonstrated that the high-pressure CO_2 extracts had activity against 13 bacteria and that better action was verified with extracts obtained at a lower CO_2 extraction density and a higher temperature.
Keywords: *Peumus boldus*; Compressed carbon dioxide; Chemical characterization; Antimicrobial activity.

INTRODUCTION

Essential oils of aromatic and medicinal plants have great potential in applications as antimicrobial agents, and their use as remedies has been recognized for a long time (Kim et al., 1995). These essential oils are in fact a complex mixture of hydrocarbons, alcohols, esters, aldehydes, carboxylic compounds and, in some cases, phenylpropanoids. The most frequently found hydrocarbons are terpenic compounds, but sesquiterpenes can also be found.

Peumus boldus Molina (Monimiaceae family) is a native herb originating in the central regions of Chile, popularly known as boldo-do-chile, or simply boldo. It is a small perennial tree, typically from arid zones, much used in popular medicine with many beneficial properties attributed to it for the treatment of biliar litiase, liver congestion, hepatic insufficiency and oxidative stress-associated diseases (Reineger et al., 1999; Jang et al., 2000). The antioxidant, anti-inflammatory and hepatoprotective effects and also teratogenic action of extracts of boldo leaves and bark have been recognized (Jang et al., 2000; Schmeda et al., 2003; Jimenez et al., 2000; Almeida et al., 2000).

In general, extension of the inhibitory effect of essential oils can be attributed to the presence of aromatic nuclei containing functional polar groups. The wide application of phenolic and chlorophenolic compounds as cleaning agents is well known in the literature and research has demonstrated that the hydroxyl group is strongly reactive, easily producing

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hydrogen bonds with enzyme active sites, which results in the inactivation of metabolic activities of microorganisms. For example, Farag et al. (1989) reported a relation between chemical profile and antimicrobial activity of some spice essential oils. Jain et al. (1993) reported on the anti-inflammatory effect of boldo extracts and their activity against some agents like *Escherichia coli*, *Neisseria gonorrheae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and also antifungal action against *Candida albicans*, *Fusarium oxysporum* and *Aspergillus niger*. Also, Vila et al. (1999) reported on the antimicrobial activity of the essential oil from the extracts of boldo leaves obtained by hydrodistillation against eleven gram-positive and gram-negative bacteria. They found that among the former, the more sensitive were *Streptococcus pyogenes* and *Micrococcus* sp., while similar activity against all of the Gram-negative bacteria was verified.

According to Katzung (2003), in biological activity studies the disk diffusion methods are satisfactory to determine the sensitivity of several microorganisms against many pharmaceutical substances and are sufficient when the resistance mechanism is due to the degradation of the antimicrobial agent by the microorganism.

In the above-mentioned work boldo extracts were obtained by extraction with conventional methods like infusion, Soxhlet and steam distillation, which have the drawbacks of the high temperatures involved and solvent residues. Extractions using supercritical carbon dioxide as solvent can be performed under mild conditions, thus reducing the risks of thermal degradation and the poor collection efficiencies of volatile analytes that can sometimes occur during the steam distillation or solvent extraction of essential oils and fragrance components (Sargenti and Lanças, 1997). Moreover, it is well known that extraction method may have a strong effect on both the qualitative and the quantitative chemical profiles of terpenic compounds found in the extracts of herbaceous matrices (Rodrigues et al., 2003, 2004).

Some work can be found in the literature concerning the use of supercritical fluid extraction of boldo to obtain alkaloids and flavonoids (del Valle et al., 2005; del Valle et al., 2004) and volatile compounds (Sargenti and Lanças, 1997). For example, Sargenti and Lanças (1997) performed extraction of boldo leaves in sequential steps using carbon dioxide modified by 10 and 30% n-hexane, 10 and 20% acetone and finally 10% methanol under only one set of experimental conditions, namely, 75°C and 80 bar. In that work however the extracts were not submitted to any test regarding the activity of the extracts obtained against microorganisms. These authors also conducted extractions by classical methods (steam distillation, Soxhlet and cold extraction) and concluded that supercritical fluid extraction was the most promising technique to extract the essential oil of boldo as it provided much better yields than steam distillation and almost all compounds extracted by the conventional methods.

Del Valle et al. (2004) studied the effect of temperature (30 to 60°C), pressure (60 to 150 bar) and solvent-to-raw material ratio (0.91 to 2.72) on the extraction yield of boldo leaves using pressurized carbon dioxide. These authors further evaluated the effect of extraction pressure (300 and 450 bar) and cosolvent concentration (2, 5 and 10% (w/w) ethanol) on the boldine (the main alkaloid in boldo leaves and bark) yield with supercritical carbon dioxide at 50°C and tested the antioxidant potency of the extracts obtained. Del Valle et al. (2005) also studied the extraction of antioxidants (mainly boldine) from boldo bark using supercritical carbon dioxide at 40°C and 400 bar and 60°C and 600 bar. Nevertheless, in neither of the above-mentioned studies is the chemical composition of the extracts obtained from supercritical carbon dioxide extraction reported, nor was their action against microorganisms studied.

In this context, the main objective of this work is to assess the effect of process extraction variables (temperature, pressure and solvent density) on the chemical profile and the antimicrobial activity of boldo leaf extracts obtained by high-pressure carbon dioxide extraction.

EXPERIMENTAL

Materials

Samples of dried boldo leaves (Laboratório Industrial Vida e Saúde LTDA, Chapecó, SC) were purchased in a natural products store in Erechim, Brazil, homogenized, crushed, classified with respect to particle size (100 to 200 mesh) and stored in a nitrogen atmosphere prior to extraction.

Apparatus and Experimental Procedure

The experiments were performed in a laboratory-scale unit, described in detail elsewhere (Rodrigues et al., 2003, 2004; Mossi et al., 2004), which consists of a CO_2 (White Martins S.A., 99.9% in liquid phase) reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 100 cm^3 jacketed extraction

vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of ± 0.31 bar, a collector vessel with a glass tube and a cold trap. Amounts of around 10 g of dried boldo leaves were fed into the extraction vessel. The CO_2 was pumped into the bed, which was supported by two 300 mesh wire disks at both ends, and was kept in contact with the herbaceous matrix for at least one hour to allow for system stabilization. Afterwards, the essential oil was collected by opening the micrometering valve and the CO_2 mass flow, kept constant at around 1 g min^{-1}, was estimated with the pump recordings. The experiments were conducted isothermally at constant pressure in approximately 5 hours of extraction. The experimental range studied was from 20 to 50°C in temperature and from 100 to 250 bar. Triplicate runs were carried out for all experimental conditions, producing an overall extraction yield standard deviation of 0.02.

Extract Characterization

The extracts were analyzed in a gas chromatograph interfaced with a mass selective detector - GC/MS (Shimadzu, Model QP 5050A), using a capillary column DB-5 (length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 μm). Helium was the carrier gas with a flow rate of 1 mL min^{-1}. An electronic impact mode of 70 eV was used. A split mode (split ratio 1:55) at 300 ºC interface temperature was used with the following column temperature gradient programming: 60°C for 2 min; 5°C min^{-1} up to 110°C, 3°C min^{-1} up to 150°C, and 15°C min^{-1} up to 300°C for 5 min. Standard samples of 20,000 ppm were prepared using dichloromethane (Merck, analytical grade) and 1 μL was injected. The 1,8-cineole, trans-sabinene, pinocarveol, pinocarvone, 4-terpineol, ascaridole, piperitone oxide, dioxide limonene and n-eicosane were identified by comparing the mass spectra obtained with those from the Wiley library. Compositions are expressed as percent of normalized peak areas.

Antimicrobial Tests

The microorganisms selected for the antimicrobial tests (antibiogram with solid disks methodology) were *Staphylococcus aureus* (ATCC 6538), *S. epidermidis* (ATCC 12228), *Sarcina* sp. (from Instituto Biológico/Campinas, SP), *Shigella flexneri* (ATCC 12022), *Shigella sonei, Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Xanthomonas campestris* (from Instituto Biológico/Campinas, SP), *Serratia marcescens* (ATCC 13880), *Aeromonas* sp. (from Instituto Biológico/Campinas, SP), *Citrobacter freundii* (ATCC 8090), *Bacillus subtilis* (ATCC 6633), *Enterobacter cloacae* (from Instituto Biológico/Campinas, SP), *Salmonella choleraesuis* (ATCC 10708), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Proteus mirabilis* (ATCC 25933) and *Enterococcus faecalis* (ATCC 19433). The bacteria were inoculated in Luria Bentani (LB**)** liquid medium (triptone 10 g L^{-1}, yeast extract 5 g L^{-1}, NaCl 5 g L^{-1}) and kept for 24 hours in an oven maintained at 37°C. Paper disks with a diameter of 7 mm were impregnated with 5 mg of boldo extracts, diffused from the disk to the plate containing the bacteria tested. A linear concentration gradient profile was observed to form from the center of the disk to the outer zone of the plates. The degree of sensitivity or resistance of a microorganism was determined by measuring the size of the zones of antimicrobial effect (size of halo formed). Samples were examined in triplicate against each bacterium together with a negative control disk and another for a positive control disk (Chloramphenicol 30 μg).

RESULTS AND DISCUSSION

Table 1 contains the results on extraction yield (mean values of three extractions) of boldo samples obtained by extraction with pressurized carbon dioxide. In this work the extraction yield was defined as the weight percentage of the extract obtained with respect to the initial charge of the raw material in the extractor and was calculated when 280 g of carbon dioxide had been consumed in the extraction. It can be observed in this table that a maximum yield of 0.38% was obtained in the extraction of boldo leaves, a result very similar compared to that obtained by del Valle et al. (2004), 0.35%, and much different from the value obtained by Sargenti and Lanças (1997), 1.12%, in their sequential extraction steps using modified carbon dioxide.

Figure 1 contains the kinetic extraction curves for the extraction of boldo leaves, where the two well-known antagonistic effects of temperature and pressure can be observed; i.e., solvent density (solvent power) at lower pressures and solute vapor pressure (temperature) at higher pressures. For example, comparing the temperature and pressure of runs 2 and 3 or runs 4 and 5, one can note that density has a positive effect on the extraction yield. For instance, at 50°C and 250 bar the extraction yield was 270% greater than that observed at the

same temperature and 100 bar. The effect of temperature on the extraction yield can be observed when runs 1, 2 and 5 are compared, for which values of solvent density are quite similar. Clearly, when the temperature is increased the extraction yield shifts to higher values. This trend can be attributed to the enhancement of solute vapor pressure and also due to an increase in the carbon dioxide diffusion coefficient and a decrease in viscosity and surface tension of the solvent, which favors the extraction process (McHugh and Krukonis, 1994; Dariva et al., 1999).

Table 1: Extraction yields of boldo leaves with compressed carbon dioxide.

Run	T (^oC)	P (bar)	Solvent density* (g cm^{-3})	Extraction yield ($g_{extract}/100g_{boldo\ leaves}$)
1	35	175	0.841	0.27 ± 0.02
2	50	250	0.835	0.38 ± 0.03
3	50	100	0.408	0.14 ± 0.01
4	20	250	0.964	0.17 ± 0.01
5	20	100	0.855	0.13 ± 0.01

*Estimated from Angus et al. (1976)

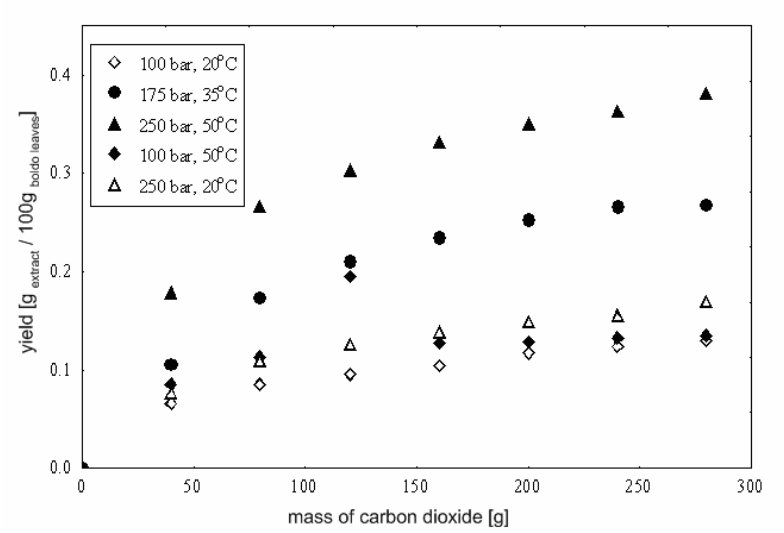

Figure 1: Kinetics of the extraction of boldo leaves with pressurized carbon dioxide.

Around 20 compounds could be identified in the extracts, but in this work, the nine representative compounds presented in Table 2 were selected for their potential application in product formulation. All analyses were replicated at least three times in order to check the experimental reproducibility and to permit statistical treatment of the results.

The compounds identified in this work differ in some aspects from those reported in the literature as several substances such as α-pinene, camphene, β-pinene, β-myrcene, thymol and methyleugenol were not verified to occur in boldo leaf extracts obtained by steam distillation (del Valle et al., 2004), hydrodistillation (Vila et al., 1999) or modified carbon dioxide extraction (Sargenti and Lanças, 1997). The composition of the extracts obtained in this work (percentage of normalized peak areas) are presented in Table 2 and compared to values in the literature; the contents of 1,8-cineole and ascaridole are higher than those reported by del Valle et al. (2004) and Vila et al. (1999). As mentioned by del Valle et al. (2004), wide compositional differences of this kind are not uncommon and divergences in chemical content of the constituents may be explained in terms of genetic variability, geographic location, harvest time, climatic conditions, cultivation handling, age of vegetable material, period and storing conditions, among others (Farias, 1999).

In Table 3 the statistical analysis of the effects of density and temperature on the content of selected compounds in boldo extracts is presented. The values presented in this table are in fact mean values of the compound concentrations for each condition. In this table the same letter between two levels of a factor

means that there is no significant difference at a confidence level of 95% ($p<0.05$) adopting the Tukey test. Inspection of Table 3 reveals that 1,8-cineole is strongly affected by temperature and density with higher contents obtained at a high temperature and a low density. An opposite trend was observed for limonene dioxide. Nevertheless, most of the compounds studied in this work did not show a significant difference in concentration within the temperature and density ranges studied.

Table 2: Chemical distribution of selected compounds present in boldo extracts obtained from pressurized carbon dioxide extraction (mean value ± standard error).

Compound	Percentage of normalized peak areas				
	35°C/175bar	50°C/250bar	50°C/100bar	20°C/250bar	20°C/100bar
1,8-cineole	4.6 ± 0.2	20.6 ± 0.1	21 ± 1	4.1 ± 0.6	8.7 ± 0.1
Sabinene	0.8 ± 0.1	0.6 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
Pinocarveol	0.8 ± 0.2	1.2 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
Pinocarvone	0.5 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.6 ± 0.2
4-terpineol	0.9 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.2 ± 0.1
Ascaridole	26 ± 1	20 ± 1	23.3 ± 0.4	23 ± 3	21.3 ± 0.6
Piperitone oxide	8.6 ± 0.8	8 ± 2	10 ± 1	14.0 ± 0.6	9.0 ± 0.5
Limonene dioxide	56 ± 3	45 ± 1	39.7 ± 0.5	51 ± 2	56.4 ± 0.1
n-eicosane	1.3 ± 0.2	1.1 ± 0.4	2.6 ± 0.5	3.7 ± 0.4	1.2 ± 0.1

Table 3: Statistical analysis of the effect of extraction temperature and density on the content of selected compounds present in boldo leaf extracts obtained by pressurized carbon dioxide extraction (composition expressed as percentage of normalized peak areas).

Compound	T (°C)			Solvent density*		
	20	35	50	low	intermediate	high
1,8-cineole	6.4^{b}	4.6^{b}	20.8^{a}	21.0^{a}	1.3^{b}	4.1^{c}
Sabinene	1.0^{a}	0.8^{a}	0.9^{a}	1.3^{a}	0.8^{b}	1.0^{b}
Pinocarveol	1.0^{a}	0.8^{a}	1.0^{a}	0.8^{a}	1.0^{a}	1.1^{a}
Pinocarvone	1.1^{a}	0.5^{b}	0.8^{b}	0.8^{a}	0.9^{a}	0.7^{a}
4-terpineol	0.5^{b}	0.9^{a}	1.0^{a}	0.8^{a}	0.8^{a}	0.9^{a}
Ascaridole	22.2^{a}	26.2^{a}	22.0^{a}	23.3^{a}	22.7^{a}	23.2^{a}
Piperitone oxide	11.4^{a}	8.6^{a}	8.9^{a}	9.7^{b}	8.6^{b}	13.9^{a}
Limonene dioxide	53.8^{a}	56.2^{a}	42.5^{b}	39.7^{b}	52.6^{a}	51.3^{a}
n-eicosane	2.4^{a}	1.3^{a}	1.9^{a}	2.6^{a}	1.2^{b}	3.7^{a}

*see Table 1.
$^{a, b, c}$ different letters represent a significant difference at 95% ($p<0.05$ - Tukey test).

The results of antimicrobial activity tests conducted on boldo extracts, expressed as the size (mm) of the inhibition halo are presented in Table 4. As mentioned above, the disk diameter was 7 mm and the presence of a halo (values greater than 7 mm) indicates some extent of antimicrobial activity. Thus, as the size of inhibition halo increases, the antimicrobial activity of the extracts is enhanced. In this table it can be observed that boldo extracts demonstrated antimicrobial activity against most of the gram-positive and gram-negative bacteria tested. The greatest antimicrobial activity of boldo extracts was verified for the gram-positive bacteria *Enterococcus faecalis* and *Staphylococcus aureus*; and for the gram-negative bacteria *Escherichia coli, Salmonella choleraesuis, Shigella sonei* and *Shigella flexneri*. The bacteria *Bacillus subtilis, Enterobacter cloacae, Proteus vulgaris, Serratia marcescens* and *Pseudomonas aeruginosa* were not present inhibited by boldo extracts.

Table 5 contains the statistical analysis of the antimicrobial tests, where a general trend of higher inhibition halo size at higher temperature and a lower density can be observed. It is worth noting that this result on antimicrobial activity may be somewhat related to that obtained for chemical composition, where the content of 1,8-cineol is also increased at a higher temperature and a lower density. No isolated test using 1,8-cineol was performed in this work, but it is well known from the literature that this compound has antimicrobial activity (Sonboli et al., 2006; Magiatis et al., 2002; Simionatto et al., 2005).

Table 4: Antimicrobial activity of boldo leaf extracts obtained by pressurized carbon dioxide extraction (mean value ± standard error).

Bacterium	ATCC	Antimicrobial activity (mm)				
		35ºC/175bar	50ºC/250bar	50ºC/100bar	20ºC/250bar	20ºC/100bar
Gram-positive						
Bacillus subtillis	6633	ns	Ns	ns	ns	ns
Enterococcus faecalis	19433	17±2	17±2	24.0±0.1	13.6±0.5	ns
Sarcina sp	*	11.0±0.7	10.0±0.2	11.6±0.5	13.6±0.5	15.6±0.5
Staphylococcus aureus	6538	16.6±0.5	17±3	16±2	13.0±0.1	12±3
Staphylococcus epidermidis	12228	10±3	13.0±2.0	13.6±0.5	11.6±0.5	9.6±0.5
Gram-negative						
Aeromonas sp	*	ns	14±1	14±1	9.6±0.5	11.0±0.1
Citrobacter freundii	8090	14±3	20±1	18.6±0.5	10±3	18±1
Enterobacter cloacae	*	ns	Ns	ns	ns	ns
Escherichia coli	25922	13±2	18±1	16±1	13.0±0.1	9.6±0.5
Klebsiella pnemoniae	13883	11±4	14±2	14.0±0.1	12.0±0.1	12.6±0.5
Proteus mirabilis	25933	ns	Ns	ns	ns	ns
Proteus vulgaris	13315	ns	Ns	ns	ns	ns
Pseudomonas aeruginosa	27853	ns	Ns	ns	ns	ns
Salmonella choleraesuis	10708	15.6±0.5	14±1	17±2	ns	ns
Serratia marcescens	13880	ns	9±2	8±1	ns	ns
Shigella flexneri	12022	16±1	14±3	16±2	11.0±0.1	11.6±0.5
Shigella sonei	*	22±1	25±2	20±1	13.6±0.5	15±2
Xanthomonas campestris	*	11±2	15±1	10±3	14±1	10±3

ATCC: American Type Culture Collection
*Obtained from Instituto Biológico/Campinas, SP
ns: nonsusceptible

Table 5: Statistical analysis of antimicrobial activity of boldo leaf extracts obtained by pressurized carbon dioxide extraction.

Bacterium	T (ºC)			Solvent density		
	20	35	50	low	intermediate	high
Bacillus subtilis	ns	ns	ns	ns	ns	ns
Enterococcus faecalis	10.3^{b}	17.0^{a}	20.5^{a}	24.0^{a}	13.7^{b}	13.5^{b}
Sarcina sp	15.0^{a}	11.3^{b}	10.8^{b}	11.5^{b}	12.3^{b}	14.5^{a}
Staphylococcus aureus	12.3^{a}	15.5^{a}	17.0^{a}	16.0^{a}	15.0^{a}	13.0^{a}
Staphylococcus epidermidis	11.0^{a}	9.5^{a}	13.3^{a}	13.5^{a}	11.0^{a}	11.5^{a}
All gram-positive	12.1^{b}	13.3^{ab}	15.4^{a}	16.2^{a}	13.0^{b}	13.1^{b}
Aeromonas sp	10.8^{b}	ns	14.0^{a}	14.0^{a}	10.7^{b}	10.5^{b}
Citrobacter freundii	14.0^{b}	14.0^{b}	19.3^{a}	18.5^{a}	17.3^{a}	10.0^{c}
Enterobacter cloacae	ns	ns	ns	ns	ns	ns
Escherichia coli	11.3^{b}	12.5^{ab}	17.0^{a}	16.0^{a}	13.3^{a}	13.0^{a}
Klebsiella pnemoniae	12.3^{a}	10.5^{a}	14.0^{a}	14.0^{a}	12.3^{a}	12.0^{a}
Proteus mirabilis	ns	ns	ns	ns	ns	ns
Proteus vulgaris	ns	ns	ns	ns	ns	ns
Pseudomonas aeruginosa	ns	ns	ns	ns	ns	ns
Salmonella choleraesuis	ns	15.5^{a}	15.3^{a}	16.5^{a}	12.2^{b}	ns
Serratia marcescens	ns	ns	8.5^{a}	8.0^{a}	ns	ns
Shigella flexneri	11.3^{b}	16.0^{a}	15.0^{a}	16.0^{a}	13.8^{b}	11.0^{b}
Shigella sonei	14.0^{b}	22.0^{a}	22.5^{a}	20.0^{a}	20.5^{a}	13.5^{b}
Xanthomonas campestris	12.0^{b}	11.0^{b}	12.5^{ab}	10.0^{b}	12.0^{b}	14.0^{a}
All gram-negative	12.2^{b}	14.5^{ab}	15.3^{a}	14.7^{a}	14.0^{ab}	12.0^{b}

ns: nonsusceptible
$^{a, b, c}$ different letters represent a significant difference at 95% ($p<0.05$ - Tukey test)

The gram-positive and gram-negative microorganisms differ in several aspects other than with respect to the structure of the cellular wall, mainly with regard to the presence of lipoproteins and lipopolysaccharides in gram-negative bacteria that form a barrier to hydrophobic compounds. According to Rang et al. (2001), these aspects have important implications in antibiotic action. In this work it was not possible to identify a general trend of inhibition of boldo extracts related to characteristics

of gram-positive and gram-negative bacteria, probably due to the polarity of the extracts obtained by high-pressure carbon dioxide extraction, which could easily cross the cell wall. In Table 5 it can be observed that higher antimicrobial effects at a low density and a high temperature were verified for groups of gram-positive and gram-negative bacteria.

According to Newall et al. (1996), the essential oil of boldo has antimicrobial activity against several microorganisms, such as *Escherichia coli, Neisseria gonorrheae, Pseudomonas aeruginosa* and *Staphylococus aureus.* In this work, the extracts of boldo had antimicrobial activity over *Escherichia coli* and *Staphylococus aureus*, but not against *Pseudomonas aeruginosa*. This fact might indicate that extraction method can affect the antimicrobial activity of the extracts, since depending on the extraction method employed, the distribution of compounds in the essential oil can vary greatly (Rodrigues et al., 2003, 2004).

CONCLUSIONS

The use of compressed carbon dioxide produced a maximum extraction yield of boldo leaves of 0.38% and an increase in temperature and density enhanced the extraction yield. With regard to chemical composition, there was the largest concentration of 1,8-cineole of the compounds quantified and it increased at a lower density and a higher temperature. The boldo extracts demonstrated good antimicrobial activity for several bacteria tested and followed the trend verified for 1,8-cineole, i.e., higher inhibition halo size at a higher temperature and a lower density. Results obtained in this work may be important to food science and technology and suggest a possible and alternative use of the extracts directly in product formulation.

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