Research, Society and Development, v. 11, n. 13, e567111335942, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i13.35942

Thermal Properties of Cinnamon (*Cinnamomum verum*) Essential Oil and Its Antibacterial Activity

Propriedades térmicas do óleo essencial de canela (*Cinnamomum verum*) e sua atividade antibacteriana

Propiedades térmicas del aceite esencial de canela (*Cinnamomum verum*) y su actividad antibacteriana

Received: 09/29/2022 | Revised: 10/10/2022 | Accepted: 10/11/2022 | Published: 10/15/2022

Lidaiane Mariáh Silva dos Santos Franciscato
ORCID: https://orcid.org/0000-0002-2724-1547
State University of Maringá, Brazil
E-mail: lidaianemariah@gmail.com
Angela Maria Ariati
ORCID: https://orcid.org/0000-0003-4726-4049
State University of Maringá, Brazil
E-mail: ang.ariati@gmail.com
Angela Maria Picolloto
ORCID: https://orcid.org/0000-0002-8969-9844
State University of Maringá, Brazil
E-mail: angela.picolloto@gmail.com
Rodrigo Zunta Raia
ORCID: https://orcid.org/0000-0003-2199-2160
State University of Maringá, Brazil
E-mail: rodrigoraia@gmail.com
Valéria Aquilino Barbosa
ORCID: https://orcid.org/0000-0003-1483-3508
State University of Maringá, Brazil
E-mail: aquilinovbarbosa@gmail.com
Paulo Rodrigo Stival Bittencourt
ORCID: https://orcid.org/0000-0001-8740-1672
Federal Technological University of Paraná, Brazil
E-mail: paulob@utfpr.edu.br
Michel Rubens dos Reis Souza
ORCID: https://orcid.org/0000-0002-9925-5008
Tiradentes University, Brazil
E-mail: michel_rubens@hotmail.com
Otávio Akira Sakai
ORCID: https://orcid.org/0000-0002-3502-5107
Federal Institute of Paraná, Brazil
E-mail: otavio.sakai@ifpr.edu.br
Elisângela Andrade Ângelo
ORCID: https://orcid.org/0000-0001-8876-4790
Federal Institute of Paraná, Brazil
E-mail: elisangela.angelo@ifpr.edu.br
Cristiane Mengue Feniman Moritz
ORCID: https://orcid.org/0000-0002-9114-2156
State University of Maringá, Brazil
E-mail: crisfeniman@yahoo.com.br

Abstract

The use of new natural antimicrobials has gained attention from the pharmaceutical and food industry, in addition to end consumers, as additive options to conventional antibiotics for resistant microorganisms and also as natural preservatives. Essential oils are secondary plant metabolites that protect plants against predators and pathogens. The aim of this study was to evaluate thermal properties of cinnamon essential oil (EO) and its antibacterial activity. Ten components were identified, with (E) - cinnamic acid (67.70%) being the major component. Cinnamon EO was thermally stable up to 106.6ºC (T_{onset}) and T_{dec} occurred at 178.5 °C and T_{offset} up to 216.0 °C. There was endothermic transition; enthalpy variation (ΔH) and activation energy (E_a) was -531.6 KJ Kg^{-1} and -1.26 ± 0.03 J mol^{-1}, respectively. Bacterial strains showed distinct resistance to tested antibiotics and variation in Minimum Inhibitory

Concentration values ranging from 0.8 to 1.6 mg mL^{-1}. Cinnamon EO initiated bactericidal effect against all bacteria tested after four hours of contact and Minimum Bactericide Concentration was 0.4 mg mL^{-1}, exception for *Bacillus cereus* (0.8 mg mL^{-1}). Analysis of cinnamon thermal properties EO showed its stable thermal performance up to 106.6 °C and broad spectrum, that may be an antimicrobial proposal.
Keywords: Food preservation; Bacterial death time; Thermal performance.

Resumo
O uso de novos antimicrobianos naturais tem ganhado atenção da indústria farmacêutica e alimentícia, além dos consumidores finais, como opções aditivas aos antibióticos convencionais para microrganismos resistentes e também como conservantes naturais. Os óleos essenciais são metabólitos secundários de plantas que protegem as plantas contra predadores e patógenos. O objetivo deste estudo foi avaliar as propriedades térmicas do óleo essencial de canela (OE) e sua atividade antibacteriana. Dez componentes foram identificados, sendo (E) - ácido cinâmico (67,70%) o componente majoritário. O OE de canela foi termicamente estável até 106,6ºC (T_{onset}) e o T_{dec} ocorreu a 178,5ºC e o T_{offset} até 216,0ºC. Houve transição endotérmica; a variação de entalpia (ΔH) e energia de ativação (Ea) foi de -531,6 KJ Kg^{-1} e -1,26 ± 0,03 J mol^{-1}, respectivamente. As cepas bacterianas apresentaram resistência distinta aos antibióticos testados e variação nos valores de Concentração Inibitória Mínima variando de 0,8 a 1,6 mg mL^{-1}. O OE de canela iniciou efeito bactericida contra todas as bactérias testadas após quatro horas de contato e a Concentração Bactericida Mínima foi de 0,4 mg mL^{-1}, exceto para *Bacillus cereus* (0,8 mg mL^{-1}). A análise das propriedades térmicas do OE de canela mostrou seu desempenho térmico estável até 106,6 °C e amplo espectro, podendo ser uma proposta antimicrobiana.
Palavras-chave: Preservação de alimentos; Tempo de morte bacteriana; Desempenho térmico.

Resumen
El uso de nuevos antimicrobianos naturales ha llamado la atención de la industria farmacéutica y alimentaria, además de los consumidores finales, como opciones aditivas a los antibióticos convencionales para microorganismos resistentes y también como conservantes naturales. Los aceites esenciales son metabolitos vegetales secundarios que protegen a las plantas contra depredadores y patógenos. El objetivo de este estudio fue evaluar las propiedades térmicas del aceite esencial (AE) de canela y su actividad antibacteriana. Se identificaron diez componentes, siendo (E) - ácido cinámico (67,70%) el componente mayoritario. El AE de canela fue térmicamente estable hasta 106,6ºC (T_{onset}) y T_{dec} se presentó a 178,5 °C y T_{offset} hasta 216,0 °C. Hubo transición endotérmica; la variación de entalpía (ΔH) y energía de activación (Ea) fue de -531,6 KJ Kg^{-1} y -1,26 ± 0,03 J mol^{-1}, respectivamente. Las cepas bacterianas mostraron una resistencia distinta a los antibióticos probados y una variación en los valores de Concentración Inhibitoria Mínima que van desde 0,8 a 1,6 mg mL^{-1}. El AE de Canela inició efecto bactericida contra todas las bacterias probadas después de cuatro horas de contacto y la Concentración Mínima de Bactericida fue de 0.4 mg mL^{-1}, a excepción de *Bacillus cereus* (0.8 mg mL^{-1}). El análisis de las propiedades térmicas de la canela EO mostró su comportamiento térmico estable hasta 106,6 °C y de amplio espectro, lo que puede ser una propuesta antimicrobiana.
Palabras clave: Conservación de los alimentos; Tiempo de muerte bacteriana; Desempeño térmico.

1. Introduction

In nature, essential oils (EOs) play an important role in protecting plants as antibacterial, antiviral, antifungal, insecticide and anti-herbivore agents. EOs can also attract some insects favoring pollen and seeds dispersion and or repelling undesirable insects (Nieto, 2017). As secondary metabolites, EOs are associated with plant's protection mechanism against predators and pathogens (Campos et al., 2016).

EOs are synthesized naturally by plants secondary metabolism and may be obtained from different plant organs, such as flowers, leaves, stems, branches, seeds, fruits, roots and barks. These components show a strong odor due to the presence of volatile compounds (Nieto, 2017), a complex mixture of compound dozens with different chemical characteristics, functional groups and polarity (Miranda et al., 2016).

Secondary metabolites classification is associated with chemical structure, composition, solubility or biosynthetic route. They are classified into three major groups: terpenoids, alkaloids and phenolics (Kabera et al., 2014). Terpenoids or terpenes are found in various plant products. Within terpenes subdivision, monoterpenes have low molecular weight, are volatile and their function in plants is to attract pollinators and repel insects (Vizzoto et al., 2016).

Due to EOs are known for playing an important role in adapting plants to environment, they also show potential sources of pharmacologically active substances (Nieto, 2017). Chemical diversity of EOs is responsible for both their aroma

presented and biological activities performance, such as antiseptics, bactericides, fungicides, antiviral (Sarto, & Zanusso Junior, 2014; Miranda et al, 2016), food preservatives, antioxidants, in addition to sanitizers application as stainless steel surfaces in food industries (Budri et al., 2015).

New natural antimicrobials use has come to the attention of food industry and final consumers. The continuous and improper use of antibiotics results in resistant microorganism's emergence to conventional antibiotics and food preservation processing. In this context, researchers, food industry and consumers started looking for alternative preservatives that can improve food safety and quality. Chemical compounds derived from natural sources may have antimicrobial properties against food borne pathogens (Gyawali, & Ibrahim, 2014). An important dietary condiment related to antimicrobial activity it exerts is cinnamon.

Cinnamon is used as a condiment in cooking (Ribeiro-Santos et al., 2017). It can be found in bark, raw or powder, widely used as a flavoring agent in food, beverages and chewing gum (Chen et al., 2014). Its leaves and bark are used as a source of volatile oils. The major component of its EO may vary depending on the part of plant and species that it has been extracted, with cinnamaldehyde being the major component of bark, leaves eugenol and bark camphor of plant's root. However, compounds variation present in cinnamon EO depends on many factors, such as variety, plants part , climatic conditions, drying conditions (if applied), extraction methods and analysis method for chemical characterization (Ribeiro-Santos et al., 2017). And physical-chemical stability and biological properties of EOs are strongly related to their chemical composition.

The aim of this study was to evaluate cinnamon thermal properties EO and its antibacterial activity.

2. Methodology

Cinnamon EO used was extracted by a 3-hour hydro distillation process in a Clevenger graduated apparatus. Cinnamon barks were obtained from the retail trade of Maringá County, Paraná State (latitude -23.415794, longitude -51.938524).

Eight bacterial cultures were used: *Bacillus cereus* INCQS 00739 (ATCC 14579), *Staphylococcus aureus* INCQS 00005 (ATCC 14558), *Staphylococcus aureus* INCQS 00381 (ATCC 29213), *Escherichia coli* INCQS 00033 (ATCC 25922), *Pseudomonas* aerugas, *Salmonella enterica* subsp. *enterica* serovar. Typhimurium INCQS 00040 (ATCC 13313), *Salmonella enterica* subsp. *enterica* serovar. Typhi INCQS 00040 (ATCC 19214) and *Shigella flexneri* INCQS 00152 (ATCC 12022) from Collection of Reference Microorganisms in Health Surveillance - CMRVS, FIOCRUZ-INCQS, Rio de Janeiro County, Rio de Janeiro State, Brazil.

2.1 Chemical inference of cinnamon EO by FTIR-ATR

Cinnamon EO spectrum sample was obtained by using FTIR-ATR technique in the region of medium infrared 4000-400 cm^{-1} in equipment model Cary 630 (Agilent). The method was described by Ruschel et al. (2014) with adaptations. The sample was spread over the surface of ATR crystal and spectrum was obtained through software Microlab Pc (Agilent). Spectra interpretations were carried out according to Lopes e Fascio (2004).

2.2 Chemical characterization of cinnamon EO by gas chromatography - mass spectrometry (CG-EM)

Chemical characterization was performed in GC-MS (Shimadzu, model GCMS-plus-QP2010), in that a capillary column DB-5 with 30 meters x 0.25 mm x 0.25 mm was used. The method was described by Beraldo et al. (2013) with adaptations. The temperature of injector and interface was 250°C. The detector was operated in Electron Impact (EI) mode at 70 eV and helium was used as carrier gas. Chromatographic conditions for cinnamon EO were an initial temperature of 40°C

(2min) at a rate of 3°C min^{-1}, heating to 250°C and maintaining this temperature for 10 minutes. A 1:10 split injection and injected volume of 1 µL of sample. A mixture of linear alkenes (C10 to C39) was injected into the chromatograph under the same conditions used as a standard for calculating retention index with linear temperature programming. The identification was performed by comparing retention index with libraries (Adams, 2017; Nist, 2019).

2.3 Thermal properties of cinnamon EO

Techniques of Thermo gravimetric Analysis (TG) were used to study the decomposition in oils. Deriving in first order TG curves, it was found the temperature of reaction beginning, T_{onset}, the temperature that decomposition was maximum T_{dec_max}, and temperature that vaporization ceases, known as T_{off_set}. Data treatment performed with TG was known as Differential Thermo gravimetric Analysis (DTG) allowing from the peak height (endothermic or exothermic) to obtain the temperatures corresponding to beginning and end of reaction with greater accuracy. The variation in vaporization enthalpy (ΔH) was obtained using thermo gram integral calculation of Differential Scanning Calorimetry (DSC) analysis and activation energy of samples was calculated by using Arrhenius method (Mianowski et al., 2014). Thermo analytical characterization was performed by simultaneous TG equipment (STA 6000 Simultaneous Thermal Analyzer Perkin Elmer Frontier). Approximately 300 µL of oils were deposited in the platinum-rhodium crucible under a dynamic N_2 atmosphere (30mL.min^{-1}), with a heating rate of 20°C min^{-1} in temperature range from 25 to 250°C. TG was used in order to provide information regarding changes in mass as a function of time and/or temperature under a dynamic atmosphere. Experiments were carried out using a thermo balance of high sensitivity, reproducibility and response to variations in mass. The equipment was previously calibrated with indium ($T_{fusão} = 156{,}6°C$), and zinc ($T_{fusão} = 420°C$).

2.4 Antimicrobial sensitivity of bacterial strains

In relation to microbial resistance characterization of bacteria under study, their anti biograms were performed with conventional antibiotics: Ampicillin (AMP), Tetracycline (TET), Gentamicin (GEN), Vancomycin (VAN), Ciprofloxacin (CIP), Clindamycin (CLI), Chloramphenicol (CLO), Azithromycin (AZI), Cefazolin (CFZ) and Amoxicillin + Clavulanate (AMC). Bacterial suspensions were prepared with *overnight* cultures in Tryptic Soy Broth (TSB) and standardized at a concentration equivalent to the 0.5 McFarland scale ($1.0x10^8$ UFC.mL^{-1}) using a spectrophotometer (Kasuaki, IL-227) with a 625nm wavelength. In Petri dishes containing Mueller Hinton Agar (MHA) culture medium, bacterial suspensions were inoculated homogeneously aiding *sterile swabs*. Antimicrobial drugs were placed on the surface of culture medium after drying the inoculums and the plates were incubated at 37°C for 24 hours to measure inhibition halos (mm) (CLSI, 2017).

2.5 Antimicrobial activity of cinnamon EO

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of EO cinnamon were determined by micro dilution method (CLSI, 2009) in micro plates, with adaptations. The concentrations of 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 mg mL^{-1} were tested.

In order to organize the highest concentration, EO cinnamon or cinnamaldehyde was diluted in equal parts of propylene glycol and subsequently in Mueller Hinton broth (MH) with 0.5% Tween 80, obtaining a double concentration. From it, serial dilution was performed on micro plate to obtain 50 µL of each concentration (columns) on micro plate wells.

Tested bacteria were activated in 3 mL of Tryptic Soy Broth (TSB) and incubated at 35°C for 24 hours. Cultures standardization was performed on the 0.5 scale of MacFarland ($1.0x10^8$ UFC.mL^{-1}) using a spectrophotometer (Kasuaki, IL-227) with 625 nm wavelength. Then, inoculum was prepared with two serial dilutions: the first one being a dilution in 1:10

proportion in saline (1.0 x 10^7 UFC.mL^{-1}) and the second being 1:10 in MH broth (1.0 x 10^6 UFC.mL^{-1}).

In micro plate, 50 µL of inoculums were added to each well, obtaining a cell concentration of 5.0 x10^5 CFU mL^{-1} and concentrations of cinnamon EO or cinnamaldehyde to be tested. Micro plates were incubated in a bacteriological oven at 37°C for 24 hours. MIC was determined with the addition of 50 µL of 0.01% resazurin indicator in each well. After two hours of incubation at 37°C, a visual stain reading was conducted, with bacterial inhibition becoming blue and bacterial growth coloring pink.

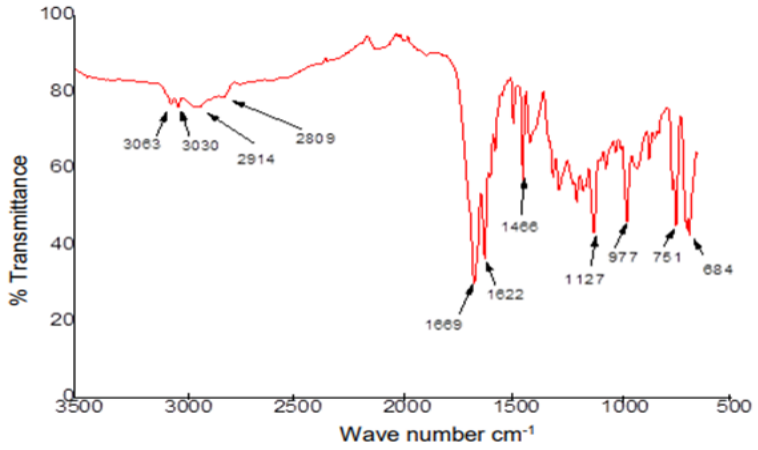
MBC verification and microbial death curve were determined by stamping each well in Petri dishes containing MH Agar, before adding resazurin to the micro plate. Plates were incubated under aerobic conditions and replicated at 2, 4, 8 and 24 hour intervals at 35°C. The non-development of bacterial colony in peaks of each well indicated bactericidal activity. All tests were performed in triplicate.

Negative controls (only MH, higher and lower concentrations of cinnamon or cinnamaldehyde EO) and positive controls (only inoculants in MH broth) were prepared. All tests were performed in triplicate. MIC and MBC of each strain were considered as the lowest concentration with the respective effect in at least two replicates.

3. Results

Figure 1 illustrates the spectrum generated in FTIR for cinnamon EO and Table 1 presents the main wave numbers, theoretical bands, functional group, vibration type and peak intensity in an organized way. Bands presence of 1669 and 1622 cm^{-1} with strong intensity, joint to band 3063 to 2809 cm^{-1} indicate the presence of carboxylic acid. The band 1466 cm^{-1} (CH_2) indicates alkenes presence. . The 1127 cm^{-1} band (CO elongation) and strong intensity are indicative of alcohol. The bands 977 cm^{-1} (CH deformation out of the plane) to 684 cm^{-1} (CH deformation out of the plane) and medium intensities may indicate benzene and derivatives, with a 1.3-di-substituted one.

Figure 1- Spectrum of samples of *Cinnamomum verum* bark essential oil.



Source: Authors based on survey data (2019).

Bands found in the FTIR spectrum of this study are in accordance with literature, confirming aldehydes presences, aromatic structures and derivatives in cinnamon EO.

Table 1. Interpretation of FTIR spectrum.

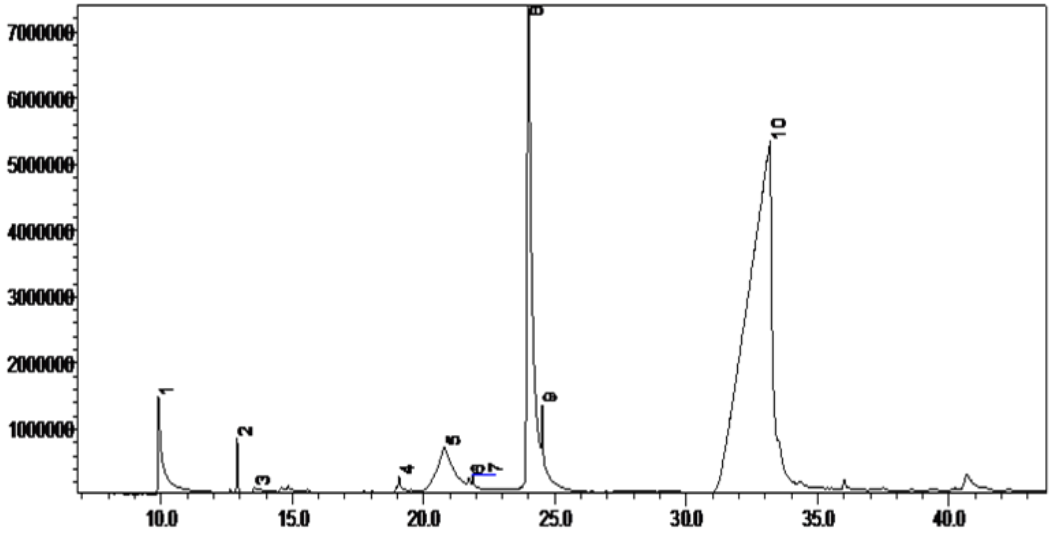
Wave Number.	Band (cm^{-1})*	Functional group	Vibration mode	Peak intensity	Organic compost
3063	3100-3000	O-H	Elongation	S	Aromatic
3030	3100-3000	O-H	Elongation	S	Aromatic
2914	3200-2500	O-H	Elongation	S	Carboxylic acid
2809	2830-2700	O-H	Elongation	S	Aldehyde
1669	1820-1630	C=O	Elongation	S	Aldehyde
1622	1680-1620	CC	Elongation	M	Alkene
1466	~1465	CH_2	Scissors deformation	M	Alkane
1127	1300-1000	C-O	Elongation	S	Alcohol
977	1000-680	C-H	Out-of-plane deformation	M	Olefin
751	770-730	C-H	Out-of-plane deformation	S	Monosubstituted benzene
684	1000-680	C-H	Out-of-plane deformation	M	Olefin

* Theoretical reference: Lopes & Fascio (2004); Frequency Intensity: S = strong, M = medium. Source: Authors based on survey data (2019).

The identification and detailed quantification of chemical compounds present in e EO was obtained through GC-MS. Compounds were listed in Table 2 and the chromatogram in Figure 2. It was possible to identify chemical classes present, such as: oxygenated monoterpenes ($C_{10}H_{18}O$), hydrocarbon sesquiterpenes ($C_{15}H_{24}$) and other oxygenated compounds.

Ten components were identified, of that *trans*-cinnamic acid (67.70%), *cis*-cinnamaldehyde (19.80%), benzenecarboxylic acid (4.60%) and benzaldehyde (3.84%) were the major components. Regarding the chemical classes, EO presented 98.89% of oxygenated compounds, followed by 0.99% of oxygenated monoterpenes and 0.12% of oxygenated sesquiterpenes.

Figure 2. Gas chromatography and mass spectrometry of cinnamon *Cinnamomum verum* essential oil, intensity (eV) versus time (min.).



Source: Authors based on survey data (2019).

Table 2. Chemical composition of cinnamon bark (*Cinnamomum verum*) essential oil.

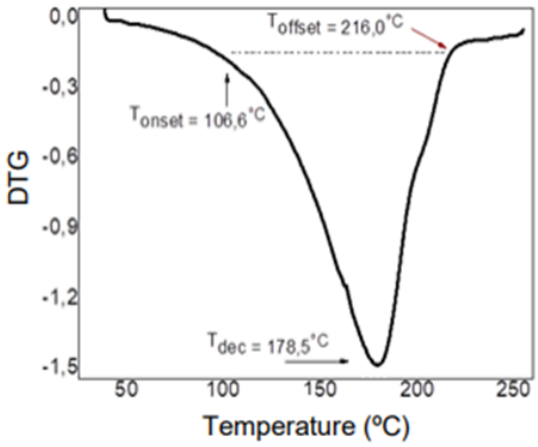
Peak	Compound[a]	Retention Ratio[b]	Relative Area (%)[c]	Formula	Identification methods
1	Benzaldehyde	963	3.84	C_7H_6O	a, b, c
2	Eucalyptol	1028	0.57	$C_{10}H_{18}O$	a, b, c
3	Phenylacetaldehyde	1043	0.19	C_8H_8O	a, b, c
4	Borneol	1164	0.33	$C_{10}H_{18}O$	a, b, c
5	Benzenocarboxylic acid	1202	4.60	$C_7H_6O_2$	a, b, c
6	*cis*-2,3-Pinanediol	1228	0.09	$C_{10}H_{18}O$	a, b, c
7	Isobornyl	1226	0.12	$C_{15}H_{24}$	a, b, c
8	*cis*-cinnamaldehyde	1274	19.80	C_9H_8O	a, b, c
9	Bornyl acetate	1286	2.76	$C_{12}H_{20}O_2$	a, b, c
10	*Trans*-Cinnamic Acid	1462	67.70	$C_9H_8\ O_2$	a, b, c

[a] Compound listed according to elution order of column DB-5; [b] Retention Index (RI) was calculated by using a homologous series of n-alkanes (C10-C39) in a capillary column (DB-5); [c] Identification based on the comparison of Nist (2019) and Adams (2017) data libraries. Relative Area (%): area percentage occupied by the compound within chromatogram. Source: Authors based on survey data (2019).

Correlating the results of GC-MS with that of FTIR, it was possible to observe that aldehyde bands identified in FTIR correspond to, *Trans*-Cinnamic Acid (67.70%), *Cis*-Cinnamaldehyde (19.80%), benzaldehyde (3.84%) identified in CG-EM. Bands with alcohol stretch correspond to chemical compound borneol (0.33%).

Figure 3 presents differential thermo gravimetric analysis (DTG) that turned it possible to identify temperatures T_{onset} (degradation onset), T_{dec} (maximum decomposition temperature, at the decomposition process that occurred more quickly) and T_{offset} (ash temperature, at that material has already been degraded) indicated by arrow.

Figure 3. Cinnamon essential oil thermogram.

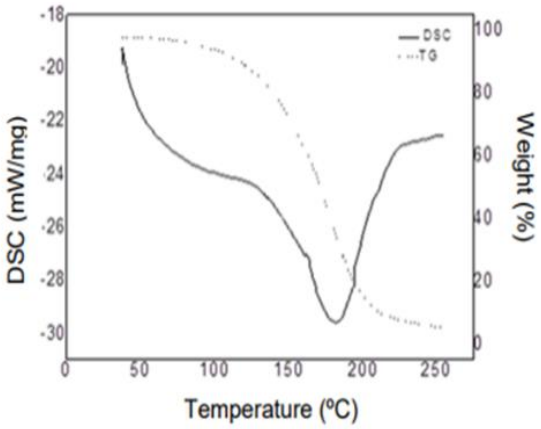


Source: Authors based on survey data (2019).

It was found that cinnamon EO was thermally stable up to 106.6 ºC (T_{onset}). Decomposition stage occurred up to 216.0 ºC and maximum decomposition temperature for this EO corresponded to 178.5 °C.

TG curve (Figure 4) showed that EO showed a single stage of decomposition with no residue at the test end, decomposition stage means total loss of mass, the same being due to EO volatilization process.

Figure 4. Differential Scanning Calorimetry (DSC) and Thermo gravimetric (TG) analysis of cinnamon essential oil



Source: Authors based on survey data (2019).

DSC thermo gram shown in Figure 4 showed an endothermic transition, with a peak temperature of 178.5 °C, referring to vaporization of cinnamic aldehyde content and other volatile compounds. Enthalpy variation ($_{\Delta}H$) was -531.6 kJ kg^{-1}. The negative sign meant the release of heat from EO into the medium. As lower $_{\Delta}H$, lower energy consumption and vaporization temperature time. There was a thermal stability of cinnamon EO up to approximately 106°C. Mass decomposition in EO showed a single stage at a certain temperature intervals. Activation energy (E_a) determined by using Arrhenius's method was -1.26 ± 0.03 J mol^{-1}.

Table 3 showed inhibition halos of conventional antibiotics against the pathogenic bacteria used in this study, as a way to characterize them for antimicrobial resistance.

Table 3- Microbial inhibition halos with conventional antimicrobials.

Bacteria	**Inhibition Halo (mm)**									
	AMP	**TET**	**GEN**	**VAN**	**CIP**	**CLI**	**CLO**	**AZI**	**CFZ**	**AMC**
Bacillus cereus ATCC 14579	15 (S)	24 (S)	26 (S)	24 (S)	37 (S)	23 (S)	31 (S)	20 (S)	25 (S)	15 (I)
Staphylococcus aureus ATCC 14558	18 (R)	9 (R)	28 (S)	22 (S)	37 (S)	25 (S)	15 (R)	25 (S)	27 (R)	25 (R)
Staphylococcus aureus ATCC 29213	20 (R)	16 (I)	16 (R)	17 (S)	6 (R)	30 (S)	25 (S)	22 (S)	27 (R)	26 (R)
Escherichia coli ATCC 25922	22 (S)	26 (S)	20 (S)	6 (RI)	35 (S)	6 (RI)	31 (R)	14 (RI)	21(S)	24 (S)
Pseudomonas aeruginosa ATCC 27853	6 (RI)	19 (S)	24 (S)	6 (RI)	30 (S)	6 (RI)	6 (R)	12 (R)	6 (R)	6 (R)
Salmonella typhimurium ATCC 13313	6 (R)	25 (S)	20 (S)	6 (RI)	30 (S)	6 (RI)	31 (S)	26 (S)	25 (S)	26 (S)
Salmonella typhi ATCC 19214	11 (R)	29 (S)	21 (S)	6 (RI)	22 (S)	6 (RI)	24 (S)	19 (I)	23 (S)	27 (S)
Shigella flexneri ATCC 12022	22 (S)	28 (S)	23 (S)	10 (RI)	30 (S)	6 (RI)	35 (S)	28 (S)	27 (S)	30 (S)

R: resistant; S: sensitive; I: intermediate; IR: intrinsic resistance. Ampicillin (AMP), Tetracycline (TET), Gentamicin (GEN), Vancomycin (VAN), Ciprofloxacin (CIP), Clindamycin (CLI), Chloramphenicol (CLO), Azithromycin (AZI), Cefazolin (CFZ) and Amoxicillin + clavulanate (AMC). Source: Authors based on survey data (2019).

Table 4 showed MIC values for cinnamon EO in relation to the bacteria tested and CBM values at different intervals of incubation time.

Table 4. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) in mg mL^{-1} of cinnamon essential oil at different incubation time intervals.

Bacteria	MIC (mg mL^{-1})	MBC (mg mL^{-1})			
	in 24 h	2h	4h	8h	24h
Bacillus cereus ATCC 14579	1.6	-	3.2	1.6	0.8
Staphylococcus aureus ATCC 14458	0.8	-	1.6	1.6	0.4
Staphylococcus aureus ATCC 29213	0.8	-	3.2	1.6	0.4
Escherichia coli ATCC 25922	0.8	-	3.2	1.6	0.4
Pseudomonas aeruginosa ATCC 27853	0.8	-	3.2	1.6	0.4
Salmonella typhimurium ATCC 13313	0.8	-	3.2	1.6	0.4
Salmonella typhi ATCC 19214	0.8	-	3.2	1.6	0.4
Shigella flexneri ATCC 12022	1.6	-	6.4	3.2	0.4

(-): bacterial growth in all tested concentrations. Source: Authors based on survey data (2019).

Cinnamon EO showed antimicrobial activity against all tested bacteria, with MIC values ranging from 0.8 to 1.6 mg mL^{-1}. In this work, cinnamon EO had a broad antimicrobial spectrum against Gram-positive and Gram-negative bacteria. Only *Bacillus cereus* and *Shigella flexneri* showed a higher MIC value. The cultures of *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 13313), *Salmonella* typhi (ATCC 19214) and *Escherichia coli* (ATCC 25922) showed the same CBM results.

4. Discussion

The results found in this study corroborate the data obtained by Jeyaratnam et al. (2016), who analyzed functional groups and chemical composition of cinnamon EO extracted by hydro distillation also through FTIR and GC/MS. Authors observed that band region between 1800 to 600 cm^{-1} is characteristic of cinnamon EO and highlighted that bands 1668 cm^{-1}, 1574 cm^{-1} and 748 cm^{-1}, 1449 cm^{-1}, 686 cm^{-1} correspond to aldehydes structures, aromatic rings, alcohol and alkenes, respectively. For compounds identification, they observed that there were four chemical classes presented: oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and other oxygenated compounds. The major compounds of each chemical group were *trans*-cinnamaldehyde (79.6%, oxygenated compounds), β-caryophyllene (3.6%, hydrocarbon sesquiterpenes), α-terpineol (1.0%, oxygenated monoterpenes) and cariophylene oxide (0.4%, oxygenated sesquiterpenes). There are not many studies in the literature that present the thermal properties of cinnamon essential oil.

However, understanding the behavior of cinnamon essential oil after variations in heat is very important as a parameter of application in processed foods, allowing to identify whether the biological properties of the oil (such as, for example, antimicrobial activity) can or cannot be changed after passing through by heat variations in the food processing steps. The decomposition temperature found in this study for cinnamon essential oil corroborates the result of Yang et al. (2021) who evaluated the thermal stability of cinnamon essential oil alone and in nanoparticles using the differential scanning calorimetry (DSC) method, which is based on measuring the heat absorbed or released by the sample during the temperature control process, and observed that cinnamon essential oil and nanoparticles have a denaturing temperature of 167.16°C and 206.61°C, respectively.

Pathogenic bacteria showed distinct resistance to tested antibiotics. *Bacillus cereus* showed inhibition halos between 15 and 37 mm, but tested antibiotics were not used as preventive substances for this bacterial culture, Batista et al. (2018) reported that antibiotic bacteriocin AS-48 (cyclic peptide) produced by *Enterococcus faecalis* bacterium had high bactericidal activity against *B. cereus* under varying temperature and pH conditions.

Pseudomonas aeruginosa showed inhibition halos between 6 and-30 mm, it was resistant to 70% of antibiotics, except tetracycline, gentamicin and ciprofloxacin, that presented inhibition halos representing sensitivity compared to antibiotic quality control Table provided by BRcast (2018). The antibiotics gentamicin and ciprofloxacin were indicated for infections treatment caused by *P. aeruginosa* and this bacterium was considered a multidrug-resistant pathogen, since it presented intrinsic resistance and ability to develop resistance mechanisms, such as mutations and acquisition of mobile genetic elements (Santos et al., 2015).

Strains of *Staphylococcus aureus* (ATCC 14458 and ATCC 29213) showed inhibition halos between 6 and 37 mm, being resistant to ampicillin, cefazolin and amoxicillin + clavulanate and equally sensitive to vancomycin, clidamycin and azithromycin. Ferreira et al. (2015) identified potentially toxigenic strains of *Staphylococcus* isolated from different critical points in a broiler slaughter unit, as well as assessed the resistance of these strains to different antimicrobials. Most isolates were resistant to sulfonamide (98.5%), nalidixic acid (89.3%) and penicillin G (87.70%). Among isolates resistant to penicillin, 4.60% were oxacillin resistant and 1.5% vancomycin resistant. Resistance to antimicrobials by *Staphylococcus* genus is associated with the existence of genes in microorganisms that encode proteins present in different biochemical mechanisms and, therefore, affect antibiotics action. This characteristic of resistance to a certain antimicrobial can extend to others, so it is important to study the susceptibility of strains to various antibiotics.

Escherichia coli ATCC 25922 strain showed inhibition halos between 6 and-35 mm, showing sensitive to ampicillin, tetracycline, gentamicin, cefazolin and amoxicillin + clavulanate antibiotics and presented intrinsic resistance to vancomycin, clindamycin and azithromycin ones. Chloramphenicol is reported in the literature as an effective antibiotic for *E. coli*. However, in this study, bacterial culture proved to be resistant to this antibiotic. Mantilla & Franco (2012) studied the antimicrobial resistance of *Escherichia coli* strains isolated from beef samples and observed that isolated strains were sensitive to gentamicin and resistant to chloramphenicol.

Salmonella typhi and *S.* typhimurium showed inhibition halos between 6 to 30 mm, showed intrinsic resistance to vancomycin and clindamycin antibiotics. Pandini et al. (2014) verified the resistance profile of different serotypes of *Salmonella* spp. isolated in broiler aviaries against 12 conventional antimicrobial agents. The highest percentage of resistance was found to tetracycline (30.8%) and the lowest to gentamicin and chloramphenicol (2.6%). These results indicated that antimicrobials should be used in aviaries more prudently in order to reduce the spread of resistant strains.

Shigella flexneri showed inhibition halos between 6 and 35 mm, had intrinsic resistance to vancomycin and clindamycin antibiotics and was sensitive to all other antibiotics tested. Jomezadeh et al. (2014) carried out a resistance study of four species of *Shigella* to conventional antibiotics and observed resistance to ampicillin and sensitivity to ciprofloxacin.

Disk diffusion method was not used to test bacteria susceptibility under study to cinnamon EO, since they are non-polar and do not easily diffuse into agar. As physicochemical properties, such as solubility, volatility and diffusion in agar (aqueous environment) of prospective herbal products are still unknown. Disk-diffusion method may not be the most suitable for comparing antimicrobial susceptibility with conventional antibiotics.

Thus, disk-diffusion method was used to have a scan of bacteria resistance under study to conventional antimicrobials, since it has the advantage of being a fast, low-cost test with dosages already established in clinical practice. In order to test bacteria susceptibility to cinnamon EO, microdilution test was chosen precisely to know MIC needed to inhibit different species. For seven bacteria, the same MIC value (0.4 mg mL^{-1}) was found, with the exception of *Bacillus cereus* (0.8 mg mL^{-}

[1]). This result found in this study predicted the broad spectrum of antibacterial action showed by cinnamon EO even against bacteria that demonstrated resistance to conventional antibiotics used in clinical practice.

Kaskatepe et al. (2016) investigated antimicrobial activity of three cinnamon bark OEs commercially obtained in Turkey (I, II and III) and two cinnamon bark OEs (IV and V) obtained on a laboratory scale (hydro distillation using Clevenger apparatus) against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213). All samples showed important antimicrobial activity and for *E. coli* values of MICs ranged from 0.09 to 12.5 mg mL^{-1} and for *S.aureus* ranged from 0.09 to 6.25 mg mL^{-1}. The highest MIC values found were from sample II 12.5 mg mL^{-1} (*E. coli*) and 6.25 mg mL^{-1} (*S.aureus*). In chromatographic study, authors observed that samples I, III, IV and V had in their composition *trans*-cinnamaldehyde as the major component in quantities of 76.9%, 99.5%, 92.3% and 90.1%, respectively, while sample II contained only 10.3%. The major component of sample II was cinnamaldehyde propylene glycol acetal with 39% of composition. From chromatography, authors found that commercial EOs did not have a natural composition of cinnamon EO, since sample III was composed by 99.5% *trans*-cinnamaldehyde; and sample I contained triacetin (22.1%) what is not a natural component of EOs and is used as a diluents or flavor carrier. Although commercial EOs presented, *trans*-cinnamaldehyde as a major compound, the other compounds were limited with benzaldehyde, benzyl alcohol or linalool, while EOs composition extracted by hydro distillation, in addition to containing cinnamaldehyde as a major compound, also contained monoterpenes or sesquiterpenes.

Zhang et al. (2016) in order to study antimicrobial activity of cinnamon EO against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) performed the determination of MIC, CBM, scanning electron microscopy and relative electrical conductivity to examine changes in permeability of membrane cell. Cinnamon EO exhibited effective antibacterial activity, with similar MIC values for both bacteria (1.0 mg mL^{-1}), while CBM was 4.0 mg mL^{-1} and 2.0 mg mL^{-1} for *E. coli* and *S. aureus*. After the addition of cinnamon EO in MIC concentration, there were changes in bacterial cells morphology, , indicating cell damage. Cells treated with concentrations of CBM were destroyed. Cinnamon EO caused leakage of small electrolytes, causing a rapid increase in electrical conductivity of samples in the first seven hours.

5. Conclusion

Antimicrobial activity determination of cinnamon EO against pathogenic bacteria associated with food throughout the production/industrialization chain, allowed proposing a natural alternative to conventional preservatives. Therefore, thermal properties analysis of cinnamon EO provided the establishment of thermal stability parameters to be considered in the processing of products involving heat. It was defined by chemical characterization that (E) - cinamic acid was the major component of cinnamon EO and it presented a broad antimicrobial spectrum and bactericidal effect after four hours of contact with bacteria. Although EO effectiveness depends on its chemical composition and target microorganism, the results presented in this study suggest that cinnamon EO may improve the action of broad spectrum, that can be an antimicrobial proposal.

References

Adams, R. P. (2017). Identification of Essential oil components by gas chromatography/mass spectrometry, 4.1rd ed, Carol Stream: Allured publishing.

Batista, R. D., Pereira, C. F., Oliveira, A. I. T., & Silva, J. F. M. (2018). Contaminação por *Bacillus cereus* e os riscos gerados através da intoxicação alimentar. *Revista Desafios*, 5, 30-40.

Beraldo. C., Daneluzzi, N. S., Scanavacca, J., Doyama, J. T., Júnior, A. F., & Moritz, C. M. F. (2013). Eficiência de óleos essenciais de canela e cravo-da-índia como sanitizantes na indústria de alimentos. *Pesquisa Agropecuária Tropical, 43*, 4, 436-440.

BrCast. (2018). Tabelas de CQ BrCAST-EUCAST. Brazilian Committee on Antimicrobial Susceptibility Testing. https:///C:/Users/Acer/Desktop/defesa/Tabelas-Controle-de-Qualidade-Rotina-e-Estendido-BrCAST-03-2016-R.pdf.

Budri, P. E., Silva, N. C. C., Bonsaglia, E. C. R., Fernades Junior, A., Araujo Junior, J. P. Doyama, J. L., & Rall, V. L. (2015). Effect of essential oils of Syzygium aromaticum and *Cinnamomum zeylanicum* and their major components on biofilm production in *Staphylococcus aureus* strains isolated from milk of cows with mastitis. *Journal Of Dairy Science*, *98*, 5899-5904. dhttps://doi.org/10.3168/jds.2015-9442

Campos, S. C., Silva, C. G., Campana, P. R. V., & Almeida, V. L. (2016). Toxicity of plant species. *Revista Brasileira de Plantas Medicinais*, *18*, 373-382. https://doi.org/10.1590/1983-084X/15_057

Chen, P., Sun, J., & Ford, P. (2014). Differentiation of the four major species of Cinnamons (*C. burmannii*, *C. verum*, *C. cassia*, and *C. loureiroi*) using a flow injection mass spectrometric (FIMS) fingerprinting method. *Journal Agricultural Food Chemical*, *62*, 2516-2521. 10.1021 / jf405580c

Clinical Laboratory Standards Institute (CLSI) (2009). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 7th. Approved standard M7-A8.

Clinical Laboratory Standards Institute (CLSI) (2017). Performance Standards for Antimicrobial Susceptibility Testing: Table 2C *Staphylococcus* spp. M02 and M07. 27 th ed. CLSI supplement M100.

Ferreira, A. A., Mendonça, R. C. S., Tette, P. A. S., Soares, A. S., & Carvalho, M.M. (2015). Identificação fenotípica e genotípica de cepas de estafilococos oriundas de uma unidade de abate de aves. *Multi-Science Journal*, *1*, 50-58. 10.33837 / msj.v1i2.85

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food Control*, *46*, 412-429. https://doi.org/10.1016/j.foodcont.2014.05.047

Jeyaratnam, N., Nour, A. H., Kanthasamy, R., Nour, A. H., Yuvaraj, A. R., & Akindoyoa, J. O. (2016). Essential oil from *Cinnamomum cassia* bark through hydrodistillation and advanced microwave assisted hydrodistillation. *Industrial Crops Products*, *92*, 57–66. https://doi.org/10.1016/j.indcrop.2016.07.049

Jomezadeh, N., Babamoradi, S., Kalantar, E., & Javaherizadeh, H. (2014). Isolation and antibiotic susceptibility of *Shigella* species from stool samples among hospitalized children in Abadan, Iran. *Gastroenterology and Hepatology From Bed to Bench*, *7*, 218–223.

Kabera, J. N., Semana, E., Mussa, A. R., & He, X. (2014). Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *Journal Pharmacy Pharmacology*, *2*, 377-392.

Kaskatepe, B., Kiymaci, M. E., Simsek, D., Erol, H. B., & Erdem, S. A. (2016). Comparison of the contents and antimicrobial activities of comercial and natural Cinnamon oils. *Indian Journal Pharmaceutical Science*, *78*, 541-548. doi: 10.4172 / ciências farmacêuticas. 1000150

Lopes, W.A., & Fascio, M. (2004). Scheme for interpreting spectra of organic substances in the infrared region. *Química Nova*, *27*, 670-673. https://doi.org/10.1590/S0100-40422004000400025

Mantilla, S. P. S., & Franco, R. M. (2012). *In vitro* microbial sensitivity profile of pathogenic *Escherichia coli* strains isolated from beef. *Colloquium Agrariae*, *8*, 10-17. https://revistas.unoeste.br/index.php/ca/article/view/360.

Mianowski, A., Tomaszewicz, M., Siudyga, T., & Radko, T. (2014). Estimation of kinetic parameters based on finite time of reaction/process: Thermogravimetric studies at isothermal and dynamic conditions. *Reaction Kinetics, Mechanisms, and Catalysis*, *111*, 45-69. 10.1007/s11144-013-0613-y

Miranda, C. A. S. F., Cardoso, M. G., Batista, L. R., Rodrigues, L. M. A., & Figueiredo, A.C.S. (2016). Essential oils from leaves of different species: antioxidant and antibacterial properties on growth pathogenic species. *Revista Ciência Agronômica*, *47*, 213-220.: https://doi.org/10.5935/1806-6690.20160025

National Institute of Standards and Technology (NIST). (2019). Nist Chemistry book on the web, SRD 69. https://webbook.nist.gov/chemistry/.

Nieto, G. (2017). Biological Activities of Three Essential Oils of the Lamiaceae Family. *Medicines*, *4*, 63,1-10. 10.3390/medicines4030063.

Pandini, J. A., Pinto, F. G. S., Muller, J. M., Weber, L. D., & Moura, A. C. (2014). Occurrence and antimicrobial resistance profile of *Salmonella* spp. isolated from aviaries in Paraná, Brazil. *Arquivos do Instituto Biológico*, *20*, 1-6. https://doi.org/10.1590/1808-1657000352013

Ribeiro-Santos, R., Andradea, M., Madella, D., Martinazzo, A. P., Moura, L. A. G., Melo, N. R., & Sanches-Silva, A. (2017). Revisiting an ancient spice with medicinal purposes: Cinnamon. *Trends in Food Science Technology*, *62*, 154-169.10.1016/J.TIFS.2017.02.011

Ruschel, C. F. C., Te Huang, C., Samiose, D., & Ferrão, M. F. (2014). Exploratory analysis applied to attenuated total reflection spectra in the Fourier Transform Infrared (ATR-FTIR) of biodiesel/diesel blends. *Química Nova*, *37*, 810-815. http://dx.doi.org/10.5935/0100-4042.20140130

Santos, I. A. L., Nogueira, J. M. R., & Mendonça, F. C. R. (2015). Antimicrobial resistance mechanisms in *Pseudomonas aeruginosa*. *Revista Brasileira Análises Clinicas*, *47*, 5-12. https://www.arca.fiocruz.br/handle/icict/15160

Sarto, M. P. M., & Zanusso Junior, G. (2014). Antimicrobial activity of essential oils. *Revista Uningá Review*, *20*, 98-102. <http://revista.uninga.br/index.php/uningareviews/article/view/1559>.

Vizzoto, M., Krolow, A. C., & Weber, G. E. B. (2016). Secondary metabolites found in plants and their importance. *Embrapa Clima Temperado*, document 316, p. 16.

Yang, K., Liu, A., Hu, A., Li, J., Zen, Z., Liu, Y., & Li, C. (2021). Preparation and characterization of cinnamon essential oil nanocapsules and comparison of volatile components and antibacterial ability of cinnamon essential oil before and after encapsulation. *Food Control*, *123*, 107783. doi:10.1016/j.foodcont.2020.10778.

Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. Y. (2016). Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. *Food Control*, *59*, 282-289. 10.17795 / jhealthscope-21808