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Research Article

Chemical profile and biological activity of clove (*Syzygium aromaticum*) extracts obtained from different extraction methods

Bruna da Silva Garais[1], Roberta Letícia Krüger[2], Leda Battestin Quast[1]*

1. Graduate Program in Food Science and Technology, Federal University of Southern Frontier, Laranjeiras do Sul, Parana, Brazil.
2. Department of Food Engineering, Midwest State University, Guarapuava, Parana, Brazil.

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Corresponding Author
Prof. Dr. Leda Battestin Quast
E-mail:
leda.quast@uffs.edu.br
Tel.: +55 42 3635-8662

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Abstract

This study aimed to evaluate the biological activity of clove (*Syzygium aromaticum)* essential oil and clove extracts, obtained by hydrodistillation, maceration, and ultrasound-assisted extraction. *E. coli*, *L. monocytogenes*, *S. enteritidis*, and *S. aureus* strains were used to determine the biological activity by the formation of inhibition zones. Eugenol (86.16 %), caryophyllene (7.14 %) and eugenyl acetate (4.84 %) were presented in clove essential oil. The ultrasound-assisted essential oils and extracts exhibited biological activity at the concentration of 100 µL/mL, with the highest inhibition halos observed for all strains studied. The essential oil presented greater inhibition for the concentration of 50 µL/mL. The chemical profile analysis demonstrated the presence of groups of gallic acid compounds, flavones, and their isomers. The total phenolic content ranged from 0.101 and 0.290 mg GAE/g, with the highest values for the ultrasound-assisted extracts. The results of DPPH varied from 8.15 to 65.24 %, with an emphasis on ultrasound-assisted essential oil and extracts.

1. Introduction

Clove (*Syzygium aromaticum)* is an aromatic plant with interest in perfume, cosmetic, health, medical, flavoring and food industries, which presents volatile compounds and antioxidants, that promotes biological activity relevant to human health [1]. The use of bioactive compounds presents great advantages over additives since they are easily decomposed, are not a pollutant, and do not present phytotoxic or residual properties. Bioactive compounds found in essential oils and plant extracts can act concomitantly with other compounds with a proven effect on pathogens [2]. The physicochemical properties and the composition of essential oils are directly affected by the extraction method. Thus, the selection of an appropriate technique is necessary to produce plant oils with high nutritional value, with no negative impact on yield and commercial value [3].

Hydrodistillation is the most widely used method for the extraction of essential oils on a laboratory scale [4]. In turn, maceration is a process widely used to obtain extracts, especially for plants with active principles susceptible to degradation at high temperatures. The ultrasound-assisted extraction generates a small amount of organic solvent residues and allows greater efficiency in the recovery of bioactive compounds from plants [5].

Spices such as cloves, cinnamon, and mint are widely used in industry as flavoring and preserving agents. Clove (*Syzygium aromaticum*) stands out from the others due to its antioxidant, antimicrobial, antiseptic, and anesthetic properties [6, 7]. Considering the potential for industrial application of clove essential oil, this study aimed to evaluate the biological activity and the chemical profile of clove essential oil and

clove extracts obtained by different extraction methods.

2. Materials and methods

Dried clove buds in natura, from a single lot, commercially produced in Brazil (Northeast Region, Latitude 13º 32' 17" S, Longitude 39º 05' 55" W and Altitude 15m) were used in this study. The clove essential oil was obtained by hydrodistillation in a Clevenger apparatus using 150 g of ground clove and 500 mL of distilled water in a 1 L round-bottom flask. The mixture was heated at 100 °C for 6 h (from boiling) in triplicate. The clove essential oil was separated manually by opening the hydrodistillation valve. Due to the density difference, the first part removed is water and then the last part is essential oil. The sample:solvent ratio and the extraction time were determined in preliminary studies.

The extracts were obtained by three methods: maceration, ultrasound-assisted extraction at 25 °C, and ultrasound-assisted extraction at 40 °C.

For the maceration procedure, 20 g of ground cloves and 80 mL of distilled water were placed in 500 mL Erlenmeyer flasks, at 25 °C. The extraction times were 0, 6, 12, 24, 48, and 72 h. The samples were stored protected from light until the moment of the filtration and then stored at 4 °C.

The ultrasound-assisted extraction (Kondortech® CD-4820) was performed in an environment at 25 ºC and 40 °C. For that, 20 g of ground cloves and 80 mL of distilled water were placed in 500 mL Erlenmeyer flasks, which were subjected to an ultrasound machine until filtration. The ultrasound exposure times were 0, 5, 10, 15, 20, and 30 min. The resulting extracts were stored at 4 °C. Due to the conditions of the equipment and time of the experiments, the maceration and ultrasound-assisted extraction tests were carried out only once.

The essential oil compounds were identified in a gas chromatograph coupled to a mass spectrometer (Shimadzu®, model GC anMS-QP2010 Ultra), equipped with a DB 5 ms fused silica capillary column (5 % diphenyl, 95 % dimethylpolysiloxane) of 30 m, with internal diameter of 0.25 mm and film thickness of 0.25 µm. Helium was used as carrier gas at a linear velocity of 43 cm/s. The operation conditions were: split mode (1:5) with an injection temperature of 210 °C; interface at 210 °C; programmed column temperature: initial temperature 50 °C held for 1 min, heating at a rate of 5 °C/min up to 130 °C, heating at a rate of 10 °C/min up to 210 °C held for 5 min. The mass spectrometer was set for 35 to 600 m/z scanning, and 2 µL of the essential oil solution diluted in n-hexane were injected, in triplicate. The compounds were identified by comparison of the mass spectrum using the NIST11 and NIST11 databases.

The extracts obtained by maceration (at 0, 6, 24, and 72 h) and ultrasound at 25 and 40 °C (at 0, 5, 15, and 30 min) were used for the characterization of the physicochemical profile by high performance liquid chromatography (HPLC). For that, 300 µL of the extract was diluted in 1 mL of distilled water. This dilution was necessary to adapt the analysis within the HPLC operating parameters. The samples were filtered through a 0.22 µm PTFE syringe filter. The analysis was conducted on a liquid chromatograph coupled to a UFLC diode array detector (Shimadzu®). A volume of 20 µL was injected, keeping the NST C18 column (250x4.6 mm, 5 µm) at 40 °C, mobile phase (phase A: 99.9 % water, 0.1 % methaneic acid; phase B: 99.9 % methanol, 0.1 % methaneic acid) with a flow rate of 1.2 mL/min, and an average run time of 30 min. The identification of the compounds (clove essential oil and extracts) was performed using a calibration curve with standard solutions of phenols ((+) catechin, (-) epicatechin, caffeic acid, vanillic acid, p-coumaric acid, trans iso-ferric acid, (-) resveratrol, myricetin, gallic acid, and quercitin). The quantification was performed by simple area normalization. The analyses were performed in triplicate at the Federal University of Southern Frontier. The sample was characterized for the moisture content, in triplicate, as described by Adolfo Lutz Institute [8]. The extraction yield of the essential oil was calculated by the ratio between the volume (mL) of essential oil and the mass (g) of raw material used [9].

The essential oil and the extracts obtained by maceration (0, 6, 24, and 72 h), and ultrasound at 25 and 40 °C (0, 5, 15, and 30 min) were used to determine the antimicrobial activity. *E. coli* (ATCC 35218), *L. monocytogenes* (ATCC 7644), *S. enteritidis* (ATCC 13076), and *S. aureus* (ATCC 25923) strains were used, from the Strain Bank of the Microbiology Laboratory of the Midwestern State University, which were cultured in tubes with 10 ml of Soy Trypticase Broth (TSB) for 24 h at 37 °C.

Bacterial suspensions were adjusted and compared to the 0.5 Mc Farland scale, and seeded in Petri plates containing 15 mL of Soy Trypticase Agar (TSA). After complete absorption of the inoculum by the culture medium, 7 mm holes were drilled, and 20 µL of the filtered extract was added. Sterile distilled water (20 µL) was used as a negative control, and the gentamicin disk was used as a positive control. The plates were kept at 4 °C for 3 h to allow diffusion of the extracts into the culture medium before microbial growth and then incubated for 24 h at 37 °C.

The antimicrobial activity was verified by the formation of inhibition halos (mm) around the holes. The assays were performed in triplicate, and the results were presented by the arithmetic mean of the inhibition halos [10].

The total phenolic content was determined as described: for the extracts obtained by hydrodistillation, maceration, and ultrasound-assisted extraction, an aqueous solution at 6 mg/mL of the dried clove extract (oven with forced and renewal air at 60 °C until constant mass) was prepared. For the essential oil, an ethanolic solution of the same concentration was prepared. In 10 mL volumetric flasks, 100 µL of each aqueous solution of the extracts was mixed with 2 mL of distilled water, 0.5 mL of Folin-Ciocalteau's reagent, and 1.5 mL of 20 % (w/v) sodium carbonate solution. The flasks were made up to 10 mL with distilled water, and stored in the dark at 25 °C for 2 h. The absorbance of the samples was measured at 765 nm in a spectrophotometer (Bel Photonics®, 2000 UV) using distilled water as a blank [11].

The results were analyzed using a standard curve of gallic acid, using 100 µL of each dilution corresponding to the concentrations of 0, 20, 30, 40, 50, 70, 90, 100, 200, 400, 600, 800, and 1000 µg/mL. The total phenolic concentration was calculated as mg gallic acid equivalent (GAE) per g of clove and expressed as mean ± standard deviation.

The essential oil and the extracts obtained by maceration (0, 6, 24, and 72 h), ultrasound at 25 and 40 °C (0, 5, 15, and 30 min) were used for the determination of antioxidant activity by the DPPH (2,2-Diphenyl-1-picryl-hydrazyl) assay, in triplicate. For the calibration curve, a Trolox 800 µmol/L solution was used and to proceed with the analyses, a solution of DPPH radical was prepared. The reading was carried out on a spectrophotometer at 520 nm [12]. The percentage of DPPH inhibition was calculated by converting to percentage of antioxidant activity (AA %) (Eq 1).

$$AA(\%) = \left[1 - \left(\frac{abs_{sample}}{abs_{blank}}\right)\right] \times 100 \quad (1)$$

where abs_{sample} is the absorbance of the sample for a given dilution, and abs_{blank} is the absorbance of the blank of the respective sample.

The results of chemical composition, antimicrobial and antioxidant analysis were analyzed using the software BioEstat 5.3®, with a confidence level of 95 %.

3. Results and discussion

The compounds identified by the chromatographic analysis (Table 1) are commonly found in essential oils [13].

Table 1. Chemical compounds of clove essential oil obtained by hydrodistillation

No.	Compound	Retention time (min)	Relative percent area
1	2-Heptanol acetate	9.80	0.27 (± 0.01)
2	Beta-cis-Ozmene	10.01	0.09 (± 0.00)
3	2-Nonanone	11.23	0.12 (± 0.00)
4	Methyl salicylate	14.20	0.28 (± 0.03)
5	Eugenol	18.74	86.16 (± 0.18)
6	Caryophyllene	19.97	7.14 (± 0.05)
7	Alpha-Humulene	20.57	0.92 (± 0.01)
8	Eugenyl acetate	21.59	4.84 (± 0.10)
9	Caryophyllene oxide	22.60	0.18 (± 0.01)

The amount and composition of essential oils can be affected by several factors such as soil, harvest time, and methods of harvesting, water stress, among others. Eugenol was the most abundant compound, with a relative percentage area of 86.16 %, followed by caryophyllene (7.14 %) and eugenyl acetate (4.84 %), which are considered typical compounds of clove essential oil [14]. These three components are the main compounds present in clove essential oil was reported by Haro-González et al., [1]. Eugenol possesses antibacterial activity, mainly on *E. coli*, *L. monocytogenes*, and *S. aureus* [15, 16], which was verified in the antibacterial activity analyses. It also has proven antioxidant activity, which reinforces the potential of the biological activity of clove oil [17].

Eugenol, caryophyllene and eugenyl acetate are volatile compounds known for their application in the

Table 2. Chemical compounds of clove extracts

No.	Compound	Retention time (min)	[M-H]+ m/z	[M-H]- m/z	Class
1	Gallic Acid	4.11	-	169	Phenolic Acid
2	Isobiflorin	8.9	-	353	Isoflavone
3	Biflorin	10.02	-	353	Flavone
4	Calicosine-glycoside	22.0	447	-	Flavone
5	Pratensein/Irilin B/Methylorobol-glycoside	23.63	463	-	Isoflavone
6	Biochanin A-7-O-glycoside	25.27	445	-	Isoflavone

Table 3. Chemical compounds of clove extracts

Sample	Mean concentration (μg/mL)		
	Gallic acid	Theobromine	Myricetin
M_{6h}	651.80^{d} (± 6.31)	0.003^{a} (± 0.000)	0.031^{d} (± 0.001)
M_{24h}	593.13^{e} (± 5.91)	0.004^{a} (± 0.001)	0.037^{abc} (± 0.001)
M_{72h}	413.53^{f} (± 8.83)	0.004^{a} (± 0.000)	0.031^{d} (± 0.001)
$U_{(25\ °C)5'}$	754.65^{b} (± 9.55)	0.006^{a} (± 0.001)	0.033^{bcd} (± 0.002)
$U_{(25\ °C)15'}$	752.64^{b} (± 3.37)	0.004^{a} (± 0.001)	0.039^{ab} (± 0.001)
$U_{(25\ °C)30'}$	654.26^{d} (± 3.48)	0.005^{a} (± 0.000)	0.032^{cd} (± 0.003)
$U_{(40\ °C)5'}$	798.58^{a} (± 4.48)	0.004^{a} (± 0.000)	0.040^{a} (± 0.001)
$U_{(40\ °C)15'}$	755.55^{b} (± 1.10)	0.005^{a} (± 0.000)	0.036^{abcd} (± 0.002)
$U_{(40\ °C)30'}$	712.71^{c} (± 2.42)	0.004^{a} (± 0.000)	0.033^{bcd} (± 0.001)

M: maceration; xh: extraction time in hours; E.g.: M0h: maceration at time zero. U: Ultrasound; (x °C): extraction temperature; x': extraction time in minutes; E.g.: U(25 °C)0': ultrasound at 25 °C at time zero. Values with the same letter in the same column are not significantly different ($p<0.05$).

pharmaceutical and chemical products, insecticides, anti-inflammatory, wound healing, anti cancer and application in food industries as baked foods, dairy products, processing foods and packaging material [1].

Compounds of the groups of gallic acid, flavones, and their isomers were detected in the chromatographic analyses (Table 2) of the extracts obtained by maceration and ultrasound, which corroborates the literature data [18]. The compounds gallic acid, theobromine, and myricetin were identified in the chromatographic analysis. As shown in Table 3, a decrease in the concentration of gallic acid was observed with increasing the extraction time, for all extraction techniques. The higher concentrations of this compound were found in the samples U(40 °C)5', U(40 °C)15', and U(40 °C)30', and the highest concentration was observed in U(40 °C)5', corresponding to 798.58 μg/mL.

Among the antioxidant compounds, polyphenols act in the sequestration of free radicals or as chelating agents of metals, and gallic acid (3,4,5-trihydroxybenzoic acid) is an example of these compounds. Scientific reports from the pharmaceutical and food industries have shown that gallic acid has antioxidant, antiviral, antibacterial, and antifungal properties [19].

No significant differences were observed for the compound theobromine among the samples, for all extraction methods, with relatively low values when compared to the other compounds. Myricetin was found in small amounts in all samples (3,5,7,3′,4′,5′-hexahydroxyflavone). It is a phenolic compound that presents antioxidant activity, pro-oxidant, phytoestrogenic effects and anticarcinogenic properties among others [20].

The moisture content of the clove sample was 14.81 ± 0.47 % which meets the identity and quality standards established by the Brazilian legislation [21], which establishes the value of 16 % as the maximum moisture allowed for all clove species.

The extraction yield (Table 4) for the essential oil was 1.05 % (on a dry basis). This value can oscillate depending on several factors, including the extraction method, extraction time, and experimental errors. Water stress in plants can impact their metabolism and cause alterations in their physiological processes, such as reduced photosynthesis and transpiration, which directly interfere with the production and composition of essential oils [22], which may explain

Table 4. Extraction yield of clove extracts obtained by maceration and ultrasound-assisted extraction at 25 and 40 °C

Sample	Yield (%)	Sample	Yield (%)	Sample	Yield (%)
Essential oil	1.05	$U_{(25\ °C)0'}$	1.45	$U_{(40\ °C)0'}$	1.45
M_{0h}	1.45	$U_{(25\ °C)5'}$	3.28	$U_{(40\ °C)5'}$	3.35
M_{6h}	6.64	$U_{(25\ °C)10'}$	4.19	$U_{(40\ °C)10'}$	4.10
M_{12h}	6.88	$U_{(25\ °C)15'}$	5.97	$U_{(40\ °C)15'}$	6.01
M_{24h}	7.26	$U_{(25\ °C)20'}$	7.33	$U_{(40\ °C)20'}$	7.26
M_{48h}	7.30	$U_{(25\ °C)30'}$	7.50	$U_{(40\ °C)30'}$	7.74
M_{72h}	7.32				

Note: M: maceration; xh: extraction time in hours; E.g.: M0h: maceration at time zero. U: Ultrasound; (x °C): extraction temperature; x': extraction time in minutes; E.g.: U(25 °C)0': ultrasound at 25 °C at time zero

Table 5. Antimicrobial activities of clove extracts at 100 µL/mL and 50 µL/mL

Sample (100 µL/mL)	Inhibition halo (mm)			
	S. enteritidis	***S. aureus***	***E. coli***	***L. monocytogenes***
Essential oil	2.40^{ab} (± 0.14)	1.85^{cd} (± 0.07)	2.10^{ab} (± 0.14)	1.75^{b} (± 0.07)
M_{24h}	0.25^{d} (± 0.00)	-	0.25^{c} (± 0.00)	-
M_{72h}	1.20^{c} (± 0.14)	1.00^{e} (± 0.14)	1.50^{b} (± 0.14)	1.10^{c} (± 0.00)
$U_{(25\ °C)15'}$	1.95^{bc} (± 0.21)	1.52^{d} (± 0.03)	1.78^{b} (± 0.18)	-
$U_{(25\ °C)30'}$	2.40^{ab} (± 0.14)	2.05^{bc} (± 0.07)	1.80^{b} (± 0.14)	1.25^{c} (± 0.07)
$U_{(40\ °C)15'}$	2.50^{ab} (± 0.14)	2.28^{ab} (± 0.04)	2.60^{a} (± 0.07)	1.88^{ab} (± 0.04)
$U_{(40\ °C)30'}$	2.92^{a} (± 0.11)	2.55^{a} (± 0.21)	2.67^{a} (± 0.03)	2.15^{a} (± 0.21)
Sample (50 µL/mL)				
Essential oil	1.25^{a} (± 0.07)	1.10^{a} (± 0.09)	1.45^{a} (± 0.07)	1.02^{a} (± 0.01)
M_{72h}	0.28^{c} (± 0.01)	-	0.30^{b} (± 0.00)	-
$U_{(25\ °C)30'}$	0.57^{b} (± 0.01)	0.52^{b} (± 0.01)	0.42^{b} (± 0.01)	0.10^{b} (± 0.00)
$U_{(40\ °C)30'}$	0.68^{b} (± 0.01)	0.42^{b} (± 0.01)	0.28^{b} (± 0.01)	0.22^{b} (± 0.01)

M: maceration; xh: extraction time in hours; E.g.: M0h: maceration at time zero. U: Ultrasound; (x °C): extraction temperature; x': extraction time in minutes; E.g.: U(25 °C)0': ultrasound at 25 °C at time zero. Values with the same letter in the same column are not significantly different ($p<0.05$).

the high moisture content and low yield observed in this study.

The extracts obtained by ultrasound and maceration showed an increase in yield with increasing extraction time. Regarding the maceration procedure, the extraction yields varied between 1.45 and 7.32 % at 0 and 72 h of extraction, respectively. For the ultrasound-assisted extraction at 25 °C, the variation was 1.45 to 7.50 % at 0 and 30 min, respectively. In the same time interval, a variation of 1.45 to 7.74 % was observed for the ultrasound-assisted extraction at 40 °C. These results are higher than that observed for the essential oil, which demonstrates an advantage of the US extraction method once it does not expose the sample to high temperatures, with no degradation of compounds of interest, besides requiring less processing time and presenting a higher yield of the final product.

The results of the antimicrobial analysis (Table 5) showed that the extracts obtained by maceration at 0 and 6 h, and by ultrasound-assisted extraction (25 °C and 40 °C) did not present inhibition halos of bacterial growth at the concentration of 100 µL/mL, indicating no detectable antimicrobial activity. The essential oils U (25 °C) 15', U(25 °C)30', U(40 °C)15', and U(40°C)30' presented the highest inhibition halos for *S. enteritidis*, between 2.40 and 2.92 mm, with no significant difference among them ($p>0.05$). Although Chesca et al., [23] reported values of 14 and 15 mm, those authors used solvents containing toxic compounds, which is not indicated for food production.

For *S. aureus*, only the treatment M24h showed no inhibition halo. The smallest halo was observed for M72h ($p<0.05$) while the other samples presented halos ranging between 1.52 and 2.55 mm. Guimarães et al., [24] reported the formation of an inhibition halo of 19 mm with the application of essential oil, while the extract exhibited no antimicrobial activity. Xu et al., [25] also verified the inhibitory activity of essential oil at concentrations of 25 % and 50 % on *S. aureus*, with inhibition halos of 16.5 and 20.4 mm, respectively, much higher than the concentration used

in this study, which suggests that higher concentrations of these extracts can lead to greater inhibition of microbial growth. The essential oils U(40 °C)15' and U(40 °C)30' showed the highest inhibition halos ($p<0.05$) for *E. coli*, between 2.10 and 2.67 mm. These values are low when compared to those found by Guimarães et al., [24], who reported a halo of 11 mm after the application of the extract; however, the authors reported no growth inhibition with the application of essential oil. For *L. monocytogenes*, the essential oils U(40 °C)15' and U(40 °C)30' presented the highest inhibition halos ($p<0.05$), between 1.75 and 2.15 mm. Silveira [26] reported that essential oil alone did not lead to halo formation for *L. monocytogenes*, but an inhibition halo of 11.8 mm was observed when a polysaccharide film was used, which highlights its potential in food preservation systems. As reported by Petropoulos et al., [22], the physiological processes of plants can be impacted by water stress, which directly interferes with the composition of essential oils and extracts, and can affect the antimicrobial capacity of essential oils and plant extracts. An antimicrobial analysis with an extract concentration of 50 µL/mL was performed with the extracts that presented antimicrobial activity at 100 µL/mL, and the essential oils showed higher antimicrobial activity for all microorganisms. It is known that eugenol, the most abundant compound in essential oils, has high antibacterial activity, especially on *E. coli*, *L. monocytogenes*, *S. typhimurium*, and *S. aureus* [15,16], which indicates that the oil acts as an effective bacterial growth inhibition agent even at lower concentrations. Therefore, essential oils are the most recommended alternative for using low bioadditive concentrations, due to their high eugenol levels.

The total phenolic content and DPPH antioxidant activity (% AA) showed antioxidant activity of the essential oils and clove extracts (Table 6). The total phenolic content ranging from 0.101 to 0.290 mg GAE/g are relatively low when compared to other essential oils and plant extracts, such as the orange pomace (Citrus sinensis) extract evaluated by Benelli et al., [27], who reported total phenolic from 9 to 36 mg GAE/g. The highest total phenolic content was found in the ultrasound-assisted extracts, which suggests the effectiveness of this extraction method.

The antioxidant activity observed for the clove essential oils and extracts ranged between 8.15 and 65.24 %, which were similar but lower when compared to the values reported by El-Maati et al., [28] in aqueous and ethanolic extracts.

Table 6. Antioxidant activity of clove extracts obtained by different extraction methods.

Sample	Total phenolics (mg GAE/g)	AA (%) (mg $_{tocopherol}$/mL)
Óleo	0.226^{e} (± 0.000)	61.93^{b} (± 0.001)
M_{0h}	0.126^{l} (± 0.001)	8.15^{j} (± 0.002)
M_{6h}	0.101^{p} (±0.000)	15.65^{h} (± 0.003)
M_{12h}	0.119^{o} (±0.001)	*
M_{24h}	0.126^{l} (±0.001)	19.95^{g} (± 0.002)
M_{48h}	0.158^{j} (±0.001)	*
M_{72h}	0.130^{k} (±0.001)	30.87^{e} (± 0.004)
$U_{(25\,°C)0'}$	0.119^{no} (±0.000)	9.47^{i} (± 0.011)
$U_{(25\,°C)5'}$	0.203^{f} (±0.001)	20.80^{f} (± 0.002)
$U_{(25\,°C)10'}$	0.188^{h} (±0.001)	*
$U_{(25\,°C)15'}$	0.196^{g} (±0.001)	32.27^{e} (± 0.004)
$U_{(25\,°C)20'}$	0.264^{b} (±0.001)	*
$U_{(25\,°C)30'}$	0.290^{a} (±0.001)	58.43^{c} (± 0.006)
$U_{(40\,°C)0'}$	0.122^{mn} (±0.001)	9.43^{i} (± 0.005)
$U_{(40\,°C)5'}$	0.118^{o} (± 0.002)	21.17^{f} (± 0.012)
$U_{(40\,°C)10'}$	0.124^{lm} (±0.0011)	*
$U_{(40\,°C)15'}$	0.162^{i} (±0.001)	40.96^{d} (± 0.005)
$U_{(40\,°C)20'}$	0.234^{d} (±0.000)	*
$U_{(40\,°C)30'}$	0.248^{c} (±0.001)	65.24^{a} (± 0.004)

* sample volume insufficient for analysis. Values with the same letter in the same column are not significantly different ($p<0.05$)

The highest antioxidant activities were observed for the essential oil and extracts obtained by ultrasound-assisted extraction, with a significant difference between the treatments. It was observed that there is a tendency for the content of total phenolic compounds to be higher in essential oil samples and in extracts obtained by ultrasound-assisted, and this tendency was observed in the largest inhibition halo for the microorganisms tested. It can be suggested that the antioxidant capacity of the extracts is related to the presence of gallic acid (Table 3), which was present in great quantities in the samples obtained using ultrasound-assisted extraction.

4. Conclusions

The extraction yields of the present study showed the advantages of ultrasound-assisted extraction, once the sample is not subjected to high temperatures, avoiding degradation of compounds of interest, besides using less processing time, with a higher yield of the final product.

For the concentration of 100 µL/mL, the essential oils and the ultrasound-assisted extracts obtained at 25

and 40 °C showed satisfactory inhibition halos for all microorganisms studied. Concerning the assay using the concentration of 50 µL/mL, the essential oil presented the highest antimicrobial activity. The chemical profile analysis of the clove extracts demonstrated the presence of compounds from the classes of gallic acid, flavones, and their isomers. Higher DPPH antioxidant activities (% AA) are observed for the essential oil and the extracts obtained by ultrasound-assisted extraction, with a significant difference when compared to the other treatments.

The results of this study have proven the biological activity of the essential oil and the extracts obtained by maceration and ultrasound-assisted extraction. The ultrasound-assisted extraction method stood out as the most recommended for the food industry due to numerous commercial advantages, including shorter processing time and lower production cost.

Abbreviations

ATCC- American Type Culture Collection

E. coli- Escherichia coli

L. monocytogenes - Listeria monocytogenes

S. aureus - Staphylococcus aureus

S. enteritidis - Salmonella enteritidis

S. typhimurium - Salmonella typhimurium

Authors' contributions

Conceptualization, formal analysis, visualization, data analysis, manuscript draft writing, writing-review & editing, B.S.G.; Conceptualization, data curation, funding acquisition, project administration, resources, supervision, roles/writing- original draft, writing-review & editing, R.L.K.; Conceptualization, project administration, resources, supervision, roles/writing-original draft, writing-review & editing, L.B.Q.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest in this study.

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