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Plant phenolic extracts as an effective strategy to control *Staphylococcus aureus*, the dairy industry pathogen

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ABSTRACT

Staphylococcus aureus is one of the most common contagious mastitis pathogens. Bovine mastitis is considered an important reservoir for dairy industry contamination, and therefore to ensure *S. aureus* control has gained a pivotal importance. Natural matrices present multiple biological effects, being its antimicrobial potential increasingly reported. Thus, the present study aims to assess the antibacterial activity of several methanol:water extracts, obtained from plants, against *Staphylococcus aureus*. Moreover, the most effective extract was characterized in terms of phenolic compounds, by using high performance liquid chromatography coupled to diode array and mass spectrometer detectors. Among the tested extracts, *Eucalyptus globulus* was the most effective against all tested *S. aureus* strains, followed by *Juglans regia* and *Foeniculum vulgare*. Inhibition halos of the plant extracts varied between 8.0–16.0 mm, excepting for *F. vulgare* in which two evident halos were observed: one with growth inhibition (5.0–7.0 mm) and a second one with visible cell density reduction (13.0–14.0 mm). Susceptibility assays evidenced that *E. globulus* extract exerted the highest antibacterial activity (MICs = 0.195–0.39 mg/mL), being effective against all the tested strains. Among the phenolic compounds identified in this extract, gallotannins, ellagic acid glycoside, and quercetin derivatives, were the most abundant; and therefore, may exert a positive and contributive effect to the observed antibacterial effect. Overall, the use of plant extracts to control bovine mastitis caused by *S. aureus* is a promising solution that could contribute to the reduction of the occurrence of dairy food industry contaminations, providing considerable benefits to agro-industries on the formulation of high-quality and safety dairy products.

1. Introduction

Bovine mastitis is the most expensive disease for the worldwide dairy industries. The management of this pathology is mainly based on the extensive use of antibiotics/disinfectants (Pieterse and Todorov, 2010), which has triggered the development of complicated scenarios of antimicrobial resistance (Motlagh et al., 2013). Beyond the poor efficacy of the antibiotic treatment, bovine mastitis has become increasingly difficult both to control and mainly to eradicate in many herds (Carter and Kerr, 2003; Sutra and Poutrel, 1994).

The increasing rates of antibiotic resistance hamper an urgent and effective bovine mastitis management, at the same time that motivate the search for effective antimicrobials (Rossi et al., 2011). Among the etiological agents for this complicated infection, *Staphylococcus aureus* is considered the most prevalent; moreover, and due to its zoonotic potential, a pivotal attention has driven an increasing solicitude by dairy industries (Kummel et al., 2016). Recently, Kummel et al. (2016) showed that *S. aureus* from bovine mastitis can enter in the dairy chain production via contaminated milk, which is in accordance with the previous study carried out by Sabour et al. (2004), who described the presence of antibiotic-resistant *Staphylococcus* species in milk processing lines, associated with chronic mastitis. Based on these findings, it becomes of the utmost importance to discover more effective, safer and selective control strategies, not only to reduce the number of microorganisms present in milking installations, but also to reduce the likelihood of bovine mastitis and milk contamination occurrence.

Natural matrices have been increasingly reported as effective alternatives to the current antimicrobial agents. In fact, the use of botanical preparation dates back from the beginning of human civilization, being effectively used in a wide variety of health conditions (Saranraj and Sivasakthi, 2014). As a rich source of bioactive molecules, among them phenolic compounds, natural matrices are commonly defined as

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"prototypes of new antimicrobial agents" (Meléndez and Capriles, 2006). Several studies have supported the effective use of botanical preparations against bovine mastitis pathogens, being aqueous and alcoholic extracts the most commonly used (Diaz et al., 2010; Doss et al., 2012; Mubarack et al., 2011; Rossi et al., 2011). However, to the authors' best knowledge no previous studies have assessed the antibacterial activity of methanol: water plant extracts against *S. aureus* isolated from bovine mastitis. In this sense, the present work aims to evaluate the antibacterial activity of fourteen methanol: water extracts against different *S. aureus* strains isolated from bovine mastitis, towards providing new insights for an effective control of dairy industry contaminations; as also to perform the characterization in terms of phenolic compounds of the most effective plant extract.

2. Materials and methods

2.1. Plant samples

A total of fourteen plant species were used: four of them were wild samples, harvested in Trás-os-Montes − Bragança, North-Eastern Portugal, namely petals of *Rosa canina* L. (rose hips/dog rose), leaves of *Juglans regia* L. (walnut), flower buds and fully opened flowers of *Rubus ulmifolius* Schott (elm-leaved blackberry), and leaves and roots of *Fragaria vesca* L. (wild strawberry). The other ones were commercial samples, namely fruits of *Pimpinella anisum* L. (anise) and *Coriandrum sativum* L. (coriander); leaves of *Melissa officinalis* L. (lemonbalm), *Eucalyptus globulus* Labill. (blue gum) and *Tabebuia impetiginosa* (Mart. ex DC) Standley (pau d'arco); aerial parts of *Foeniculum vulgare* Miller (fennel), *Matricaria recutita* L. (chamomile) and *Echinacea purpurea* (L.) Moench (purple coneflower), and lastly flowering parts of *Pterospartum tridentatum* (L.) Willk (carqueja). Plant scientific nomenclature is according The Plant List (2013), version 1.1 (2013).

2.2. Standards and reagents

Acetonitrile (99.9%, HPLC grade) and methanol (99%, PA) were from Fisher Scientific (Lisbon, Portugal). Phenolic standards were from Extrasynthèse (Genay, France). Formic acid was from Sigma-Aldrich (St. Louis, MO, USA). Tryptic Soy Broth (TSB) and Agar were purchased from Liofilchem (Roseto degli Abruzzi, Italy) and Merck (Darmstadt, Germany), respectively. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Carrollton, USA).

2.3. Preparation of the extracts

Methanol: water extracts were obtained by extracting each plant sample (1 g) with 30 mL of methanol: water (80:20, v/v) mixture at 25 °C and 150 rpm for 1 h, and filtering through Whatman No. 4 paper. Final residue was then extracted with an additional 30 mL portion of the methanol: water mixture. Each one of the combined extracts was evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and then lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). The lyophilized methanol: water extracts were re-dissolved in water to obtain stock solutions at 50 mg/mL, from which several dilutions were prepared.

2.4. Evaluation of the antibacterial activity

2.4.1. Disc diffusion assay

Seven *S. aureus* strains were used in this study (Table 1), one from the American Type Culture Collection (ATCC 25923), and six clinical isolates from cows with mastitis (North region of Portugal). The clinical isolates were provided by Segalab (Laboratório de Sanidade Animal e Segurança Alimentar, S. A.).

All strains were inoculated into 15 mL of TSB from Tryptic Soy Agar (TSA) plates not older than 2 days, and grown for 24 h at 37 °C in an orbital shaker at 120 rpm. Cells were harvested by centrifugation (for 5 min at 9500*g* at 4 °C), resuspended in TSB, adjusted to an optical density (640 nm) equivalent to 1×10^6 cells/mL and, then used in the subsequent assays. An aliquot of each strain (300 µL) was spread in TSA plates. An aliquot (25 µL) of each plant extract, with a known concentration (50 mg/mL), was placed on a sterile blank disc. Sterile water was used as negative control. Then, plates were incubated at 37 °C, during 24–48 h, and the determination of inhibitory effects was performed measuring the corresponding zones of inhibition (inhibition halo diameter).

2.4.2. Determination of minimal inhibitory concentrations (MICs)

Minimal inhibitory concentrations (MICs) were determined by microbroth dilution technique, to the plant extracts in which most pronounced effects were observed, considering the results obtained in the disc diffusion assay. MIC values were determined to the selected plant extracts by serial two-fold dilutions method, at concentrations ranging from 0.049 mg/mL to 6.25 mg/mL, adjusting final cellular concentration to 5×10^5 cells/mL. The 96-wells plates (Orange Scientific, Braine-l'Alleud, Belgium) were incubated at 37 °C for 24–48 h. Sample and bacteria-free controls were also included. After visualization of the resultant plate, MIC values corresponded to the concentration used in which no visible growth was observed, or a bacteriostatic effect was observed by comparison with positive controls (cells grown without extracts). Then, the number of viable cells was assessed by determination of the number of colony forming units (CFUs), after 24 h of incubation at 37 °C. The number of colonies formed was counted and the results presented as the total of CFUs (Log CFUs). Experiments were carried out in triplicate, and repeated in three independent occasions.

2.5. Phenolic compounds analysis

The most effective extract was characterized regarding its phenolic composition. Therefore, it was re-dissolved at a concentration of 5 mg/mL with 80% methanol, filtered through a 0.22-µm disposable LC filter disc before the chromatographic analysis. The phenolic profile was determined by HPLC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA), following a procedure previously described by Bessada et al. (2016). Detection was achieved with DAD (280, 330 and 370 nm as preferred wavelengths) and in a mass spectrometer (MS). The MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source. The identification of the phenolic compounds was performed using standard compounds, when available, by comparing their retention times, UV–vis and mass spectra; as also, comparing the obtained information with available data reported in literature giving a tentative identification. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of the most similar available standard, such as for compounds. The results were expressed as mg per g of extract.

2.6. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and means were compared using Tukey's honestly significant difference (HSD) multiple comparisons test. All statistical tests were performed using Prism software package (GraphPad Software version 6.0 for Macintosh). Results were considered statistically significant when $P < 0.05$.

Table 1
Antibacterial activity of the hydromethanolic extracts from different plant origin, against *Staphylococcus aureus* isolates.

Plant extracts	Inhibition zones (mm)						
	S. aureus ATCC 25923	*S. aureus* 1	*S. aureus 2*	*S. aureus 3*	*S. aureus 4*	*S. aureus 5*	*S. aureus 6*
Coriandrum sativum L.	–	–	–	–	–	–	–
Echinacea purpurea (L.) Moench	–	–	–	–	–	–	–
Eucalyptus globulus Labill.	10	12	10	8	14	11	15
Foeniculum vulgare Miller	5/13 [a]	6/14 [a]	5/14 [a]	6/14 [a]	7/13 [a]	5/14 [a]	6/14 [a]
Fragaria vesca L. leaves	6	6	7	6	9	6	7
Fragaria vesca L. roots	6	6	6	6	9	5	8
Juglans regia L.	14	15	14	13	12	16	16
Matricaria recutita L.	5	4	4	5	9	6	6
Melissa officinalis L.	8	9	8	8	9	10	8
Pimpinella anisum L.	–	–	–	–	–	–	–
Pterospartum tridentatum (L.) Willk.	5	5	6	3	7	5	5
Rosa canina L.	–	–	–	–	–	–	–
Rubus ulmifolius Schott	8	8	9	8	9	10	10
Tabebuia impetiginosa (Mart. ex DC) Standley	–	–	–	–	–	–	–

(–) Absence of antibacterial activity.
[a] Inhibition zone/cell density reduction.

3. Results

3.1. Antibacterial activity

Table 1 shows the obtained results for the screening of antibacterial activity, using disc diffusion assay. Among the tested plant extracts, *E. globulus* and *J. regia* revealed to be the most effective against most of tested strains, and the inhibition zones varied, respectively, between 8 and 15 and 12–16 nm. For *F. vulgare* extract a significant effect was also observed, being evident two different halos: one of complete growth inhibition − weak halo (5–7 mm), and another with visible cell density reduction − strong halo (13–14 mm). These results suggest that *F. vulgare* extract mainly exerted bacteriostatic effects. On the other hand, for *M. officinalis* and *R. ulmifolius* extracts only moderate halos were observed (ranging from 8 to 10 mm), while weak inhibition zones were observed in *M. recutita*, *F. vesca*, and *P. tridentatum* extracts, being stated tenuous variations according the tested *S. aureus* strains. Finally, at the tested concentration (50 mg/mL), no positive results were observed in *C. sativum*, *E. purpurea*, *P. anisum*, *R. canina* and *T. impetiginosa* extracts.

Subsequently, aiming to deepen knowledge and to confirm the results obtained, for plant extracts which evidenced the most pronounced antibacterial potential, namely *E. globulus*, *J. regia* and *F. vulgare* extracts, antimicrobial susceptibility tests (MIC and CFUs determination) were performed, and the results obtained presented in Table 2 and Fig. 1. Regarding the results obtained in the susceptibility tests, it was evident the bactericidal effect of *J. regia* and *E. globulus* extracts, causing a full growth inhibition of *S. aureus* strains, while for *F. vulgare* extract only bacteriostatic effects were reached against all tested strains. However, among the three tested extracts, *E. globulus* clearly evidenced to be the most effective, presenting the lowest MIC value (0.19-0.39 mg/mL), followed by *J. regia* (0.78–1.56 mg/mL) and, then *F. vulgare* extracts (3.125 to > 6.25 mg/mL). After 24 h-incubation, a half MIC value of *E. globulus* extract could decrease by 65–85% (0.5–0.8 log reduction) the viability of all tested strains ($P < 0.05$), while at concentrations higher than MIC were sufficient to full inhibit *S. aureus* growth (Fig. 1).

3.2. Phenolic compounds analysis

Rosa canina (Guimarães et al., 2013b), *Juglans regia* (Santos et al., 2013), *Rubus ulmifolius* (Martins et al., 2014a), *Fragaria vesca* (Dias et al., 2015), *Pimpinella anisum* (Martins et al., 2016), *Coriandrum sativum* (Martins et al., 2016), *Melissa officinalis* (Barros et al., 2013), *Foeniculum vulgare* (Caleja et al., 2015), *Matricaria recutita* (Guimarães et al., 2013a), *Echinacea purpurea* (Pires et al., 2016) and *Pterospartum tridentatum* (Roriz et al., 2014) extracts were previously chemically characterized by our research group. In the present study, we performed the characterization of the most active extract. Thus, the phenolic profile of *E. globulus*, obtained after methanol/water extraction, and recorded at 280 nm is shown in Fig. 2; peak characteristics and tentative identities are presented in Table 3. Nineteen phenolic compounds were detected, sixteen of which were flavonoids (mainly quercetin derivatives) and three phenolic acids (mainly gallic and ellagic acids derivatives). Compounds 3 (5-*O*-caffeoylquinic acid), 11 (quercetin-3-*O*-glucuronide) and 12 (quercetin-3-*O*-glucoside) were positively identified according to their retention times, mass spectra and UV–vis characteristics in comparison with commercial standards. Compounds 1 and 2 ($[M-H]^-$ at *m/z* 483) were identified as digalloyl-glucoside based on its fragmentation pattern, showing two product ions at *m/z* 313 (loss of a galloyl moiety) and at *m/z* 169 (deprotonated gallic acid), as already reported by Santos et al. (2011). Compounds 4, 6 and 7 ($[M-H]^-$ at *m/z* 635) were identified as trigalloyl-glucoside, corresponding to the loss of a galloyl residue as reported by Boulekbache-Makhlouf et al. (2013) in *E. globulus* leaves. In the same way, compounds 9 and 10 ($[M-H]^-$ at *m/z* 787) were assigned as tetragalloyl-glucose and compound 14 ($[M-H]^-$ at *m/z* 939) as pentagalloyl-glucoside (Boulekbache-Makhlouf et al., 2013).

Compounds 8 and 18 presented a UV spectra and a fragmentation pattern corresponding to ellagic acid derivatives. Compound 8 ($[M-H]^-$ at *m/z* 463) presented a fragmentation pattern yielding a product ion at

Table 2
Antibacterial activity of the tested hydromethanolic plant extracts, against *S. aureus* isolates, determined by CLSI broth microdilution method.

MIC (mg/mL)							
Plant extracts	*S. aureus* ATCC 25923	*S. aureus* 1	*S. aureus 2*	*S. aureus 3*	*S. aureus 4*	*S. aureus 5*	*S. aureus 6*
Juglans regia L.	0.78	1.56	1.56	1.56	1.56	0.78	1.56
Eucalyptus globulus Labill.	0.19	0.19	0.19	0.39	0.19	0.19	0.39
Foeniculum vulgare Miller	> 6.25	6.25	6.25	> 6.25	6.25	3.125	> 6.25

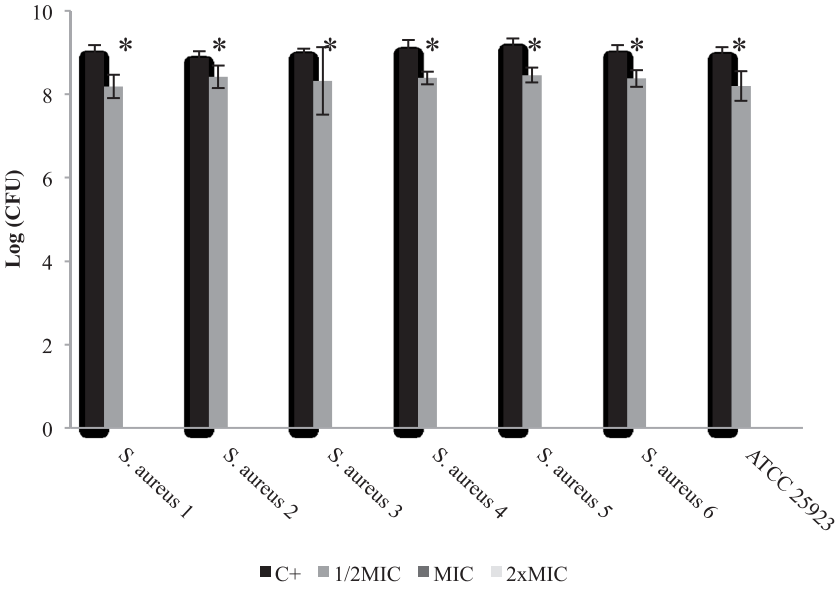

Fig. 1. Colony forming units (CFUs) of different *Staphylococcus aureus* strains, cultured within different concentrations of the hydro-methanolic extract of *Eucalyptus globulus* Labill. The means ± standard deviations for three independent assays are illustrated. $P < 0.05$ (one-way ANOVA, Tukey's multiple comparisons test).

m/z 301 (−162 u, loss of a glucoside moiety), being assigned to an ellagic acid glucoside. Compound 18 ([M-H]$^-$ at *m/z* 447) fragmented at *m/z* 315 (−132 u, loss of pentosyl) and at *m/z* 301, corresponds to methylellagic acid pentoside; this compound has been previously reported in *E. globulus* leaves by Boulekbache-Makhlouf et al. (2013) and *E. globulus* bark by Santos et al. (2011). Compound 13 ([M-H]$^-$ at *m/z* 497) yielded fragment ions at *m/z* 169 and 313, with the loss of 184 u characteristic of oleuropeic acid (Hasegawa et al., 2008), being coherent with the structure of eucaglobulin or globulusin B, which have already identified in leaves and fruits of *E. globulus* (Boulekbache-Makhlouf et al., 2013; Hasegawa et al., 2008).

The remaining compounds were identified as flavonoid derivatives. Compound 5 ([M-H]$^-$ at *m/z* 493) was assigned to myricetin-*O*-glucuronide, being already identified in different extracts obtained from *Eucalyptus* species (Santos et al., 2012). Compounds 15 ([M-H]$^-$ at *m/z* 433) and 16 ([M-H]$^-$ at *m/z* 447) with an unique MS^2 fragment ion at *m/z* 301 (loss of a pentosyl moiety −132 u and rhamnosyl moiety −146 u, respectively), were assigned to quercetin derivatives, namely quercetin-*O*-pentoside and quercetin-*O*-rhamnoside. Compound 17 ([M-H]$^-$ at *m/z* 461) was assigned to isorhamnetin-*O*-rhamnoside taking into account the fragmentation pattern described by Santos et al. (2011) for *Eucalyptus* species. Finally, no definite identification could be provided for compound 19 ([M-H]$^-$ at *m/z* 547), thus being assigned as a quercetin derivative.

Overall, among the nineteen phenolic compounds identified, di-galloyl-glucoside, 5-*O*-caffeoylquinic acid and ellagic acid glucoside, were the most abundant molecules present in *Eucalyptus globulus* methanol: water extract.

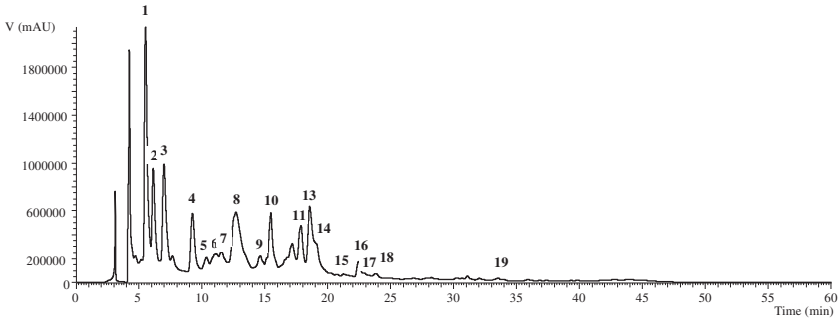

Fig. 2. Phenolic profile of *Eucalyptus globulus* Labill. methanol/water (80:20, v/v) extract at 280 nm.

4. Discussion

To overcome the problem of antibiotics/disinfectants resistance, effective and safer alternatives to the current antimicrobial drugs need to be urgently assessed, towards bovine mastitis occurrence prevention, while major triggering factor for contamination in dairy food industries. Several studies have reported the use of plant extracts as effective antimicrobial agents, using different extraction solvents, but the assessment of methanol: water extracts efficiency still continues being scarce. For example, Martins et al. conducted an experiment using *Origanum vulgare* L. (Martins et al., 2014b) and *Thymus vulgaris* L. (Martins et al., 2015a) aqueous and methanol: water extracts against a wide variety of bacteria, including *S. aureus*. However, up to the tested concentration (20 mg/mL), no positive results were achieved by the authors. Further, in the present study the selected plant species were already reported by Martins et al. (2015b) as having a promisor antifungal activity against *Candida* species. In that experiment, the authors emphasized the doubtless importance of extraction solvents used; in fact, the content in bioactive molecules, such as phenolic compounds is markedly affected by the type of extraction solvent used, since it affects phytochemicals solubility and consequent bioactivity (Martins et al., 2015a, 2015b). In this sense, based on the previous results obtained by the authors, in the present study fourteen plant extracts were screened for its antibacterial activity against six different *S. aureus* strains isolated from bovine mastitis and one reference strain.

Among the studied plant extracts, *E. globulus* revealed to be the most effective against all strains tested, presenting the higher inhibition halo and lower MIC values. In fact, whenever it is intended to select an antimicrobial drug, MIC value must be taken into consideration, since a lower MIC value indicates that a lower concentration of drug is

Table 3
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of the phenolic compounds present in the methanol/water extract of *Eucalyptus globulus* Labill.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Quantification (mg/g extract)
1	5.51	276	483	331(24),313(21),271(100),211(7),169(5)	Digalloyl-glucoside	30.5 ± 1.2
2	6.15	272	483	331(30),313(19),271(100),211(10),169(7)	Digalloyl-glucoside	13.7 ± 0.1
3	6.98	328	353	191(100),179(22),161(5),135(5)	5-*O*-Caffeoylquinic acid	22.3 ± 0.3
4	9.25	277	635	483(17),465(100),313(12),211(5),169(3)	Trigalloyl-glucoside	12 ± 1
5	10.36	340	493	317(100)	Myricetin-*O*-glucuronide	1.90 ± 0.03
6	10.84	277	635	483(100),465(10),331(5),313(7),271(5),211(5),169(3)	Trigalloyl-glucoside	6.1 ± 0.2
7	11.54	278	635	483(100),465(44),313(11),211(6),169(3)	Trigalloyl-glucoside	5.8 ± 0.2
8	12.71	253/ sh360	463	301(100)	Ellagic acid glucoside	21.6 ± 0.3
9	14.64	276	787	635(28),617(31),483(84),465(100),447(6),423(73),313(10),271(9),169(5)	Tetragalloyl-glucose	5.8 ± 0.3
10	15.47	277	787	635(37),617(22),483(55),465(100),447(62),423(15),313(7),271(5),169(3)	Tetragalloyl-glucose	11.6 ± 0.5
11	17.89	355	477	301(100)	Quercetin-3-*O*-glucuronide	7.7 ± 0.2
12	18.45	350	463	301(100)	Quercetin-3-*O*-glucoside	3.7 ± 0.1
13	18.81	283	497	313(49),169(100)	Eucaglobulin/ Globulusin B	13.9 ± 0.4
14	19.12	277	939	787(100),635(22),617(28),465(33)	Pentagalloyl-glucoside	6.5 ± 0.1
15	21.26	354	433	301(100)	Quercetin-*O*-pentoside	1.34 ± 0.01
16	22.44	354	447	301(100)	Quercetin-*O*-rhamnoside	3.2 ± 0.1
17	23.29	358	461	315(100)	Isorhametin-*O*-rhamnoside	1.16 ± 0.02
18	23.82	250/ sh364	447	315(100),301(28)	Methylellagic acid pentoside	3.2 ± 0.1
19	33.52	355	547	463(47),301(100)	Quercetin derivative	1.47 ± 0.02
					Total phenolic compounds	173 ± 4

necessary to inhibit microbial growth, being also the infectious agent less resistant to the tested drug (Dafale et al., 2016). As mentioned before, the MIC value of *E. globulus* could promote 100% of growth inhibition. These results were similar to those obtained by Rossi et al. (2011) with hexane extracts of *H. nymphoides* and *S. auriculata*. Even though not tested directly against bovine mastitis-related strains, the results obtained by Martins et al. (2015b) using these plant extracts against different *Candida* species, highlight the broad-spectrum activity of methanol: water extracts, which is in accordance with the results obtained in the present study. This achievement revealed to be a crucial advantage, since in bovine mastitis several etiological agents (bacterial and mycotic mastitis) are involved.

Several plant-derived phenolic extracts were previously reported as having prominent antimicrobial effects. *Senna macranthera* (Coll.) H. S. Irwin & Barneby var. *macranthera* (ethanol extract), *Artemisia absinthium* L. (dichloromethane extract), *Cymbopogon nardus* (L.) Rendle (80% ethanol/water extract) and *Baccharis dracunculifolia* D.C. (ethanol extract) extracts were markedly highlighted as effective antimicrobials in the management of this pathology (MIC values varied from 0.5–1.0 mg/mL) (Diaz et al., 2010). On the other hand, Rossi et al. (2011) also assessed the antimicrobial potential of several plant extracts from aquatic origin, namely *Salvinia auriculata* Aubl. and *Hydrocleys nymphoides* (Willd.) Buchenau, against bovine pathogens. The authors observed that hexane extracts were the most active preparations, ranging MIC values from 0.2 to 1.0 mg/mL (Rossi et al., 2011). Despite the promissory results obtained by the authors, the use of this organic solvent present some drawbacks, such as environmental and adverse health effects (Tanzi et al., 2012). Further, Mubarack et al. (2011) and Doss et al. (2012), evaluating the antimicrobial effect of different plant extract preparations (aqueous and alcoholic extracts) against several mastitis pathogens, also found very interesting results: inhibition zones varied from 8.0 to 25.0 mm, and the corresponding MIC values from 0.125 to 2.0 mg/mL. All the previously mentioned plant extracts were already tested against *S. aureus*, being the results achieved similar those of the present work. Moreover, despite the difference in the extraction solvents used, the authors highlighted that the intramammary use of the tested plant extracts (mainly aqueous extract − infusion) might constitute an upcoming antimicrobial agent, used alone or even combined with antibiotics, aiming to ensure an effective treatment of bovine mastitis.

In fact, natural matrices have been increasingly recognized as a rich source of bioactive molecules, among them phenolic compounds with pronounced antioxidant and antimicrobial effects, being even reported their useful incorporation in new antimicrobial products development (Mordmuang et al., 2015). Since *E. globulus* was the most effective plant extract tested, its phenolic characterization was performed aiming to determine which phenolic compounds were responsible for the observed antibacterial activity. Gallotannins, ellagic acid glycosides and quercetin derivatives were the most abundant phenolic compounds present in the *E. globulus* extract, and might be involved in the positive effects observed. These phytochemicals were already recognized as antimicrobial compounds namely against *S. aureus* and in some cases against biofilms cells, being possibly responsible for the bioactivity presented by *E. globulus* leaves hydroalcoholic extracts (Al-Zahrani, 2012; Amin et al., 2015; Fontaine et al., 2017). Thus, the results obtained drew attention for the possible use of *E. globulus* and *J. regia* extracts in combination with other drugs, both to control bovine mastitis and even dairy food industry contaminations. In fact, through using a proper combination strategy, it will be possible to achieve a greater efficacy (synergy) using lower doses of chemical drugs, also reducing the development of microbial drug resistance and preventing side effects occurrence. On the other hand, these results also highlight the possible use of these natural antimicrobial agents as disinfectants for surfaces cleaning and disinfection, with the objective of ensuring a better control of pathogenic microorganisms, responsible for bovine mastitis and dairy food industry contaminations.

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