Title: **Effect of Erica australis extract on CACO-2 cells, fibroblasts and selected pathogenic bacteria responsible for wound infection**

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Abstract

Plants from the genus *Erica* are used in many countries to treat several ailments. In this work we intend to evaluate the potential *in vivo* benefits of *Erica australis* L. by testing *in vitro* the effect induced by the plant extract when in contact with BJ fibroblasts (3 and 6 hours) and Caco-2 cells (3, 6 and 24 hours). Effects on five pathogenic microorganisms (*Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*) were also determined. It was found that the extracts enhanced fibroblast proliferation (maximum of 484% of control at 6 hour exposure) while Caco-2 cells viability was reduced in a concentration and time dependent manner (minimum of 22.3% of control at 24 hour exposure). Antimicrobial effects were also detected, with differences registered among the plant parts and solvent used, with the lowest minimum concentration for diffusion inhibition (MCDI) of 1 mg/mL. Results obtained with the fibroblasts and bacteria strongly show that this plant has potential to be used in wound healing as a stimulant of fibroblast growth and disinfection, as well as an antibiotic. Results obtained with Caco-2 cells indicate this plant also has some potential for and application as anticancer agent.

Keywords: *Erica australis* L., Fibroblasts, Caco-2, antiproliferative effect, wound healing stimulant, antibacterial

1. Introduction

Plant extracts have been used for a long time by traditional healers and are referenced in folk medicine to treat several ailments. This knowledge was somewhat lost due to the rise of allopathic medicine which replaced natural treatments with chemicals and drugs (Neves,

et al., 2009). This change, however, led to antibiotic resistant bacteria and a weakened immune system (Alanis, 2005). In addition, changes in lifestyle lead to a much larger exposure to oxidizing agents, which can cause problems that include degenerative diseases, heart problems and cancer (Ames, et al., 1995; Jemal, et al., 2008).

For this reason focus started to shift, turning the attention back to the study of plants and use of natural compounds for the treatment of diseases which led to extensive screenings for plants that could be used (Adetutu, et al., 2011b; Matkowski & Piotrowska, 2006; Neves, et al., 2009; Nunes, et al., 2012). One of the most common aspects studied in plants is their content in antioxidants, which can help in the prevention of cancer and degenerative diseases (Ames, et al., 1995). This antioxidant protection can be due to several actions, including the capture of Reactive Oxigen Species before they interact with lipids by direct scavenging of the radicals, the inhibition of enzimes such as cyclooxygenase and lipoxygenase or the stimulation of detoxifying enzymes such as indoles (Ames, et al., 1995). Some plants however, in addition to free radical scavenging properties, also possess compounds that evidence cytotoxic properties. This may not only allow them to help preventing cancer occurrence, but potentially eliminating it (González-Sarrías, et al., 2012).

The plants here studied belong to the *Ericaceae* family and are used in many countries to treat several ailments, including digestive and urinary disorders, as well as wound healing and disinfection (Akkol, et al., 2008; Harnafi, et al., 2007; Neves, et al., 2009). Aqueous extracts from two species of this plant, *Erica australis* L. and *Erica arborea* L., were previously studied by our group and were found to possess relatively high activities, especially concerning the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical (IC_{50} of 60 μg/mL) (Nunes, et al., 2012). The sample with the best results was further studied and it was found that leaves contained almost 50 mg of amino acids per gram of dry weight, of

which 20 mg are of essential amino acids. They also possess a total of 16 phenolic compounds, including the strong antioxidant caffeic acid (500 μg/g) (Nunes & Carvalho). Now, continuing and extending the study of this plant, we intended to evaluate potential health benefits derived from the consumption/direct application of extracts from this plant, by analyzing the extract effect on fibroblasts (responsible for tissue regeneration), Caco-2 cells (intestinal cancer cells) and pathogenic bacteria commonly found in wounds. The selected bacteria were *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*. *E. faecalis* is a pathogenic microorganism responsible for 90% of the infections caused by the *Enterococcus* genus (F.D.A., 2012). These bacteria are amongst the most common bacteria isolated from infected surgical sites (Munaff, 2012). Pathogenic *E. coli* species can be fatal and also commonly found in infected wounds (F.D.A., 2012; Senthil Kumar, et al., 2006). *B. cereus* can in some cases enable further contamination by other bacteria and is high prevalent in post-operative and post-traumatic wounds (F.D.A., 2012). *S. aureus* are also highly prevalent in infected wounds. Deaths are rare and only occur in people with compromised immune system (F.D.A., 2012). *L. monocytogenes* can provoke a severe form of infection with death rate between 15 and 30%, although if it triggers meningitis it can reach 70% (F.D.A., 2012).

2. Material and methods

2.1. Plant material

Samples were identified by a botanist and randomly selected and collected during the blooming period at the end of spring, beginning of summer of 2010 from the Algarve region (37.187596,-8.695455). A voucher specimen was deposited in the Herbarium from

University of Algarve with the number 10945. After collection, plant materials were stored on a dry place, protected from sunlight and naturally air dried (ambient temperature of approximately 20 ºC) for about one week. Leaves and flowers were manually separated and stored on plastic vials at -20 ºC until extraction.

2.2. Biological material

Cell lines BJ (human fibroblasts) – ATCC CRL-2522(Rockville, USA) and Caco-2 (human colon adenocarcinoma) – ATCC HTB-37, bacteria strains: *Enterococcus faecalis* – DSMZ 20478, *Bacillus cereus* – ATCC 11778, *Escherichia coli* – ATCC 8739, *Staphylococcus aureus* – ATCC 6538, *Listeria monocytogenes* – DSMZ 7644. Bacteria strains were gently provided by Stress by Antibiotics and Virulence of Enterococci laboratory from IBET.

2.3. Chemicals

Phosphate buffer saline (PBS) tablets pH 7.4, Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin at +10,000 units/mL/+10,000µg/mL, nonessential amino acids (N.E.A.A.), L-glutamine 200 mM, trypsin–EDTA (ethylenediaminetetraacetic acid) solution (2.5 g/l trypsin, 0.5 g/l EDTA), trypan blue solution (0.4%), thiazolyl blue tetrazolium bromide (MTT) and sodium dodecyl sulphate (SDS) were purchased from Sigma Chemicals, Germany. Dimethyl sulfoxide (DMSO), chloramphenicol blue, Tris-acetate-EDTA (TAE) buffer ampicillin, plate count agar, RINGER tablets and cetrimide agar were purchased from Merk, Germany. Methanol was purchased from VWR, Pennsylvania, USA. Luria broth was purchased from Sigma-Aldrich Co. Ltd, Poole, UK. Ethanol was purchased from Merck, Nottingham, UK. Discs for minimum concentration

for diffusion inhibition (MCDI) assay were purchased from Whatman, Maidstone, UK. FBS was purchased from Gibco, Invitrogen, USA. All reagents were of analytical grade.

2.4. Extraction procedure

The extraction was carried in a Soxhlet device with Electrothermal heating mantles (Electrothermal, Essex, UK), using 0.4 g of plant material and 60 mL of water or methanol. When the extraction was finished, the methanol was evaporated (Nahita serie 503, Navarra, Spain) and the material was re-suspended in water. Extracts were transferred to Eppendorfs and stored at -20 ºC until next day. On the day of analysis, extracts were put on ice and in the dark until unfrozen.

2.5. Cell lines culture

The Caco-2 (HTB 37) and BJ (CRL-2522) cells were used between passages 65-80 and 20-30, respectively. Both cells were grown in flasks in a humidified 5% CO_2/95% atmospheric air incubator at 37 °C. The cell culture medium for Caco-2 cells was DMEM supplemented with 10% FBS, 1% L-glutamine, 1% non-essential amino acids and 1% of penicillin/streptomycin. Medium was exchanged every 2-3 days and cells were sub cultured weekly. BJ cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin and cells were sub cultured 3 times a week.

2.6. Bacterial strains culture

Prior to the assay, an overnight culture of each microorganism was initiated in 5 mL Luria Broth and incubated at 37 ºC for 24 hours. After the 24 hours, a streak was made in a petri dish with 20 mL of dry plate count agar medium, and grown at 37 ºC for 24 hours.

145

146 2.7. MTT assay

147 Taking into consideration the optimal confluence of culture and sufficient medium
148 nutrients, cells were seeded in 96 well plates i) at a density of 2.5×10^4 cells/well in 200 µl
149 of medium for BJ cells and ii) at a density of 1×10^4 cells/well in 100 µl of medium for
150 Caco-2 cells. Cells were then incubated overnight until the assay.

151 For the BJ cells, the medium was replaced by fresh medium after the overnight incubation,
152 containing additionally MTT (5 mg/mL in PBS, pH 7.4). Dry methanol leaf extracts were
153 previously added to the MTT to test the extract influence. Following incubation (3 and 6
154 hours), medium was removed and DMSO was added to each well to dissolve MTT
155 formazan generated during incubation. The absorbance was measured at 540 nm (reference
156 wavelength was 690 nm) using a microplate reader (Multiscan GO, Thermo Scientific)
157 USA (Carmichael, 1987). Cells incubated in cell culture medium were considered as a
158 control for 100% cell viability.

159 The assay was performed on three occasions with six replicates at each concentration of test
160 substance in each instance. All results are, therefore, means from triplicates and are
161 presented as % of cell viability ± SD.

162 For the Caco-2 cells, the medium was also removed after incubation and replaced by 100
163 µL of aqueous leaf or flower extracts diluted in complete DMEM without FBS
164 (concentrations of 0.1, 0.5, 1.0 and 2.0 mg/mL). After incubation with the extracts for 3, 6
165 or 24 hours, MTT was added and incubated for 3 hours. After that time, DMSO was used to
166 dissolve formazan crystals and the absorbance was measured using a microplate reader
167 (Tecan, Infinite M200, Austria) as referenced above. Due to the strong color of the extracts,

an intermediate step of washing with PBS prior to MTT addition was introduced. Cells incubated with cell culture medium were considered as the control for 100% cell viability. The assay was performed on three occasions with six replicates at each concentration of test substance in each instance. All results are means from triplicates and are presented as % of cell viability ± SD.

2.8. Disc diffusion assay

For the determination of minimum concentration for diffusion inhibition (MCDI) a method similar to that used in antibiotic susceptibility testing was used, but with plant extracts instead (Gaudreau & Gilbert, 1997). Briefly, a grown microorganism colony was collected and suspended in a flask with Ringer solution. An aliquot of 50 μL was spread on a petri dish containing plate count agar. After dried, discs were put in the petri dish and 10 μL of extract (10 mg/mL) were added to each one. Ampicillin (10 mg/mL) was used as control. This procedure was repeated for every strain. After 48 hour incubation, the inhibition diameter was measured. After assessing which strains were inhibited by the extract, several concentrations were tested to find the MCDI. All results are means from two measurements of each disc, three discs per plate triplicates ± SD.

2.9. Statistical methods

Statistical analysis of fibroblast cell and Caco-2 cells culture cytotoxicity experiments was performed by ANOVA followed by Duncan's post hoc test.

3. Results

3.1 Caco- 2 cells viability

192 The influence of aqueous extract exposure in Caco-2 cells was analyzed. Two different
193 plant parts (leaves and flowers) and three different time points (3, 6 and 24 hours) were
194 tested at 0.1, 0.5, 1.0 mg/mL for the 3 and 6 hour points and 0.5, 1.0 and 2.0 mg/mL for the
195 24 hour point (Figure 1).
196 After incubating the cells with the extract, the amount of MTT formazan generated by the
197 tricarboxylic acid cycle decarboxylases in mitochondria of viable, biochemically active
198 cells, was analyzed. This reflects metabolic activity of the cells and, as such, their viability.
199 As shown in Figure 1, at 3 hours there was an increase in the metabolic activity of cells
200 upon contact with the extracts of both parts of the plant, an effect that increased in a
201 concentration dependent manner. Upon 6 hours of continuous exposure there was still an
202 increase for both plant parts, but of lower intensity (data not shown), suggesting that a more
203 prolonged contact could result in a different effect. For this reason a time point of 24 hours
204 was tested. This exposure induced a major decrease in metabolic activity, which resulted in
205 a major decrease in cell viability, compared to that of the control. This decrease led to
206 values 50% below that of the control, for a minimum concentration of 1 mg/mL and
207 confirmed the previous suggestion of a time-dependent behavior. In general, the response
208 between both parts of the plant was similar in all conditions ($p < 0.05$) with a decrease in
209 metabolic activity when the concentration increased. There was however an exception,
210 registered when the cells were exposed for 24 hours to the leaves aqueous extract at a
211 concentration of 2 mg/mL. In that case, the metabolic activity of cells increased when
212 compared to a concentration of 1.0 mg/mL ($p < 0.05$). Additionally, the result was
213 statistically different comparing with the flower aqueous extract ($p < 0.05$), which
214 continued to decrease. The highest cytotoxic effect (22% metabolic activity comparing with
215 the control) was registered for the flower extract at a concentration of 2 mg/mL, followed

by the leaves extract at 1 mg/mL (34% metabolic activity comparing with control), showing a very strong effect.

3.2 Fibroblasts proliferation

The influence of dry methanolic leaf extracts of *Erica australis* L. on fibroblasts proliferation was studied, testing different extract concentrations (Figure 2). The obtained results evidence, at first, that the tested extracts were not toxic to BJ fibroblasts, as cell viability was in all cases above 100%. In fact, upon both 3 hours and 6 hours incubation there was a significant increase in the metabolic activity, as evidenced by an increase in absorbance, which demonstrates the stimulation effect of the extract. This effect was observed for the range of concentrations between 0.5 and 2.0 mg/mL (3 hours exposure) ($p < 0.05$), and 0.5 and 1.5 mg/mL (6 hours exposure) ($p < 0.05$). At this time point (6 hours) the metabolic activity remained constant when a higher concentration of the extract (2 mg/mL) was used, suggesting that the stimulation reached the plateau at 1.5 mg/mL. The effect on the metabolic activity after 6 hours of incubation was significantly higher than after 3 hours ($p<0.05$), unlike the observed for Caco-2 cells, where the extract started showing a different trend after 6 hours of exposure.

3.3 Antibacterial effect

To ascertain if the extracts had antimicrobial properties the disc diffusion method was used, which is commonly applied to evaluate microorganism susceptibility to antibiotics. The extract at different concentrations is put in a porous paper disc. The exposure of the bacteria to the extract is accomplished via diffusion. It is verified if there is bacterial growth around the disc (Figure 3). The minimum concentration of extract needed to prevent the growth of

240 the bacteria in these conditions can then be determined (Table I). Two plant parts (leaves
241 and flowers) and two extract types (aqueous and methanolic later evaporated and
242 resuspended in water) were tested using a panel of foodborne 5 microorganisms, 3 of which
243 are commonly found in wound infections, but all of them can contaminate wounds and
244 cause an infection. It was found that the microorganisms responded differently, according
245 to plant part and extract type used, which indicates the compounds with antimicrobial
246 activity differ from plant part to plant part. Aqueous leaves extract was the one showing
247 better results, evidencing some activity against all the tested microorganisms. It was
248 followed by the aqueous flower extract, which had effect in 3 out of 5 microorganisms and
249 finally both methanolic extracts, which had effect in only 2 out of the 5 microorganisms.
250 Not only the leaves extract was the one that had effect on more microorganisms, it also
251 provided the strongest effects.
252
253 **4. Discussion**
254 One common *in vitro* assay to determine the cytotoxicity of plant extracts involves Caco-2
255 cells (Grey, et al., 2010; Russo, et al., 2005). These cells belong to a cell line derived from
256 human colon tissue and were used in this study to evaluate the effects of the plant extract in
257 human colon cancer cells (Grey, et al., 2010; Ren, et al., 2003; Russo, et al., 2005). The
258 observed effect evolved in a time dependent manner, mostly dependent on the
259 concentration of the extract used. This is a common behavior that is observed for the
260 majority of conditions. Except at the concentration of 2.0 mg/mL after 24 hours exposition,
261 all results were similar in both leaves and flowers, which means both plant parts can be
262 used when attempting to extract compounds with potential cytotoxic effect, with similar
263 results up to a concentration of 1.0 mg/mL. At a concentration of 2 mg/mL, however, the

flowers showed better cytotoxic ability than leaves, which can be due to some synergetic effect that manifests itself when the concentration of the extract is increased. Leaves on the other hand follow an opposite behavior, with a reduction of the cytotoxic effect at 2 mg/mL, perhaps due to an antagonistic effect caused by some compound present on the extract. The results obtained in this study for 24 hour exposure to the extract are higher than those obtained with wheat upon 24 and 96 hours exposure (Okarter, 2011) and with two types of cabbage at 24 and 48 hour exposure (Zarzour, 2011), but lower than those obtained with *T. gallica* upon 72 hour exposure (Boulaaba, et al., 2013). Although the obtained results show the potential of Erica australis as a cytotoxic agent, it has to be pointed out that in a normal ingestion of this plant infusion, the individual will not benefit from these properties, as according to the obtained results it takes over 6 hours of contact with the extract for the effect observation. After this time however, the effects can lead to a high reduction of the metabolic activity, so the use of this plant should not be discarded, but rather improved.

A wound occurs when the epithelial integrity is broken, which can affect the structure of the adjacent tissues. Quick wound healing depends on factors such as the proliferation of fibroblast cells and the prevention of an infection (Enoch & Leaper D., 2005). Infections occur when microorganisms contaminate and grow in the wound (Senthil Kumar, et al., 2006), an effect that hampers the healing process (Enoch & Leaper D., 2005; Senthil Kumar, et al., 2006; Shrivastava, 2011). For this reason it is important that not only the regeneration of the tissue is pursued, but also that this tissue is maintained free of infections. This has potentiated the research on the study of the application of plant extracts as antimicrobial agents (Adetutu, et al., 2011b; Raja, et al., 2011; Steenkamp, et al., 2004), an event of increased importance nowadays that bacteria resistance to antibiotics is a matter

of concern. Finding suitable therapeutic alternatives has become a major priority. In this work, we evaluated the ability of plant extracts to enhance fibroblast activity in order to determine the *in vitro* wound healing potential. The extract effect on 5 microorganism strains commonly found in wound infections was further determined to assess the *in vitro* disinfection capacity and potential application in a wound treating product.

The results obtained in the study strongly indicate that *E. australis* might be a potential candidate for dermal wound healing, because of its proliferative effect on fibroblast cells and the antibacterial activity.

An explanation for such positive effect in fibroblasts can be the high and diverse composition of antioxidants evidenced by *E. australis* L., as preliminary studies have revealed (Nunes, et al., 2012). It is known that various plant extracts abounding in antioxidants are useful in prevention or treatment of skin disorders, especially those mediated by UV irradiation. Reactive oxygen can cause harmful effects in keratinocytes and fibroblasts if antioxidative defense mechanisms are exhausted (Adetutu, et al., 2011a). Many different compounds have been tested alone or in combination (betacarotene, ascorbic acid, tocopherol, selenium and polyphenols) for prevention of sunburn, photodermatoses and photocarcinogenesis with divergent results (Tebbe, 2001). Different plant products are considered potential agents for wound healing and this kind of natural therapy is largely preferred because of the widespread availability, non-toxicity on skin cells, ease of administration and effectiveness even as crude preparations. In addition, because of the high concentration of caffeic acid in the investigated plant material, which is well known as a protector of human skin against UVB-induced erythema, *E. australis* has the potential to be used as a main compound on photoprotective cosmetics (Nunes, et al., 2012; Svobodová, et al., 2003).

312 The only extract that had effect on *E. faecalis* and *E. coli* was the leaves aqueous extract,
313 with a MCDI of 6 mg/mL in both cases. This relatively high MCDI and resistance to
314 extracts was expected because these microorganisms are known to be resistant to a wide
315 range of antibiotics (Munaff, 2012). The fact that only the aqueous extract had an inhibitory
316 effect shows that the compound(s) responsible for the effect are only present in leaves and
317 can be extracted with water but not with methanol. This hints at their polarity and is
318 important information if compound extraction and isolation is to be pursued to achieve a
319 more concentrated solution with higher activity. It is possible that the compounds
320 responsible for the activity against *E. faecalis* and *E. coli* are the same, which might be
321 explained by the presence of both bacteria in the digestive system, as inhabitants, although
322 one is Gram $^{-}$ while the other is Gram $^{+}$.
323 The growth of *B. cereus* and *S. aureus* was inhibited by all the tested extracts. Of these two,
324 *B. cereus* was more susceptible than *S. aureus*, and more so to aqueous extract of leaves;
325 the other extracts showing no difference among them. In the case of *S. aureus* a difference
326 was seen according to the solvent used and not to the plant part in study. These results
327 show that both plant parts being tested possess compounds responsible for preventing the
328 growth of these bacteria and they can be extracted with water or methanol, meaning the
329 extraction of compounds for a possible use does not need to be selective. This can be due to
330 an intermediate polarity of the compounds or presence of some compounds that can be
331 extracted with water and some with methanol. They also show that the compounds
332 responsible for the inhibition are different or, at least, there are more compounds with that
333 effect in the extract, when comparing to those that have antimicrobian activity against *E.*
334 *faecalis* and *E. coli*.

335 *L. monocytogenes* growth was inhibited by both aqueous extracts but not by the methanolic
336 extracts and more so by the leaves. These results show that while both leaves and flowers
337 possess compounds capable of preventing this microorganism growth, it is not possible to
338 extract them with methanol, indicating the polarity of the compounds. A similar trend was
339 observed with *E. faecallis* and *E. coli*, where although leaves had compounds with
340 antimicrobial activity, only water was able to extract them.
341 Overall, the results show that both plant parts and solvent used had influence on the
342 antimicrobial activity. The combinations that yield a stronger antimicrobial activity are
343 leaves extracted with water followed by flowers extracted with water. Methanolic extracts
344 had a much lower activity, with the exception of when put into contact with *Bacillus cereus*
345 where they performed similarly to flower aqueous extract. According to Fabry et al (1998),
346 crude solvent extracts of plants are considered potentially useful in therapeutics if they have
347 concentrations of inhibition values < 8mg/mL, which means all tested extracts are
348 potentially useful. This is especially true for leaves extract and especially against *B. cereus*
349 and *S. aureus*. Much lower MCDIs can be obtained if the particular compound that has the
350 inhibitory effect can be isolated, which may be worthwhile to pursue considering the effects
351 of some of the analyzed bacteria. Another important aspect relates to wounds. It was
352 previously found that the extracts have potential to be used to treat wounds given the
353 enhanced proliferation of fibroblasts. In addition to that, the fact that the extracts have some
354 inhibitory effects against microorganisms found commonly in infected wounds, increases
355 the potential use of this plant in treating wounds and preventing their infection. It also
356 supports the traditional use of this plant as a wound treating agent.
357
358 **5. Conclusion**

With this study we can conclude that the use of *Erica australis* in traditional medicine with the aim of wound healing is somewhat supported, since this plant extracts potentiated the proliferation of fibroblast cells. In addition, the plant shows antimicrobial activity against microorganisms commonly found in wound infections, and as such can serve a dual purpose in wound treatment. This activity was in some cases solvent and plant part dependent. While this plant displayed a cytotoxic effect in Caco-2 cells, this took over 6 hours to be evident, which makes challenging its use in the ambit of cancer therapy. However, the effects should not be underestimated since after 24 hours of exposition the cell metabolic activity was greatly diminished.

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454 Figure Captions

455 Figure 1. Metabolic activity (as % of control) of Caco-2 cells upon exposure to the samples
456 for 3 hours and 24 hours exposure (mean ± SD, n = 3). Differences between plant parts ($p < 0.05$)
457 are marked with *.

458

459 Figure 2. Fibroblast BJ cells metabolic activity (as % of control) with 3 hours and 6 hours
460 exposure (mean ± SD, n = 3). Differences between times ($p < 0.05$) are marked with *.

461

462 Figure 3. Inhibition of *B. cereus* by 4 plant extracts at 10 mg/mL (1- Aqueous leaf; 2-
463 Aqueous flower; 3- Methanolic leaf and 4- Methanolic flower) and by Ampicilin (A)

Table I. Microorganism inhibition by plant extract (mean ± SD, n = 3)

Extract	Parameter	*Microorganism*				
		E. faecalis	*B. cereus*	*E. coli*	*S. aureus*	*L. monocytogenes*
Aqueous leaf	Inhibition (mm) at 10 mg/mL	8.0 ± 0.0	9.0 ± 1.0	8.0 ± 0.0	8.0 ± 0.0	10.0 ± 0.0
	MCDI (mg/mL)	6.0	1.0	6.0	2.0	5.0
Aqueous flower	Inhibition (mm) at 10 mg/mL	n.i.	8.0 ± 0.0	n.i.	7.0 ± 0.0	9.3 ± 0.6
	MCDI (mg/mL)	n.i.	2.0	n.i.	2.0	8.0
Methanolic leaf	Inhibition (mm) at 10 mg/mL	n.i.	10.0 ± 0.0	n.i.	8.3 ± 0.6	n.i.
	MCDI (mg/mL)	n.i.	2.0	n.i.	4.0	n.i.
Methanolic flower	Inhibition (mm) at 10 mg/mL	n.i.	10.0 ± 0.0	n.i.	9.3 ± 0.6	n.i.
	MCDI (mg/mL)	n.i.	2.0	n.i.	4.0	n.i.
Ampicillin	Inhibition (mm) at 10 mg/mL	20.0 ± 0.0	18.0 ± 0.0	32.0 ± 0.0	50.0 ± 1.0	42.0 ± 1.0

n.i. – no inhibition