

Full Length Research Paper

Antimicrobial activity of different *Aloe barbadensis* Mill. and *Aloe arborescens* Mill. leaf fractions

Marco Pellizzoni¹, Gabriela Ruzickova², Libor Kalhotka³ and Luigi Lucini^{1*}

¹Institute of Environmental and Agricultural Chemistry, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy.

²Department of Crop Science, Breeding and Plant Medicine, Mendel University in Brno, 613 00 Brno, Czech Republic.

³Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Mendel University in Brno, 613 00 Brno, Czech Republic.

Accepted 11 January, 2012

The interest on *Aloe* based natural products is fast growing during recent years, particularly regarding their nutraceutical and antimicrobial properties. However, the information available on this item is limited and discrepancies can be found in literature. In this work, the antimicrobial properties of *Aloe barbadensis* Miller and *Aloe arborescens* Miller dermal and inner gel leaf fractions on the growth of five microorganisms were studied. Generally, gram positive were more susceptible than gram negative microorganisms and a lower activity of all fractions against the beneficial *Saccharomyces boulardii* was observed. Albeit the antimicrobial effect of pure anthraquinones aloin and aloe emodin was confirmed, a higher activity was observed for the methanol/ethyl acetate extracts of epidermis and for the water extract of the gel. A synergistic effect of several compounds, additional to the anthraquinones considered, should therefore be supposed. Conclusively, the *Aloe* extracts had an antimicrobial activity which is strongly related to the plant species and leaf fraction considered and which is a function of the microorganism considered.

Key words: Anthraquinones, acemannan, diffusion disc method, tandem mass spectrometry.

INTRODUCTION

The genus *Aloe* L. counts over 300 species and several of them are cultivated or used due to their important medicinal, industrial and cosmetic properties. Basically, all the species have similar constituents, however, *Aloe barbadensis* Miller (often called *Aloe vera* L.) and *Aloe arborescens* Miller are the most extensively cultivated in the world (Liao et al., 2006).

The *Aloe* leaf should be divided into two main fractions, namely the outer green rind and the inner colourless parenchyma. The rind is rich in 1,8-dihydroxyanthraquinone derivatives and their glycosides (Hamman, 2008), while the parenchyma tissue or pulp is rich in complex carbohydrates among which a partially acetylated mannan (acemannan or carrysin) seems to be the most bioactive compound. Among the anthraquinones,

aloin A and B (collectively known as barbaloin) are the most relevant, and are substantially located in the outer green rind. However, acemannans are composed of a glucose and mannose chain with β -(1-4) bounds and galactose branching. They are concentrated in the inner gel of leaves and have variable molecular weights, ranging from 80 kDa to over one million Da (Hamman, 2008; Eshun and He, 2004; Boudreau and Beland, 2006).

The antimicrobial properties of *Aloe* and use of their derivatives are reported in the literature, however with many discrepancies among authors, probably because of the different plant fractions considered or due to differences in geographical location of the plants, to different species of *Aloe*, to seasonal changes, to different extraction and processing procedures. It is well known, however, that the physical and chemical properties and the activity of natural products are a function of the type of extract, species of plant and the raw materials utilised (Moody et al., 2004; Rajurkar et al., 2001). Anthraquinones showed antimicrobial activity

*Corresponding author. E-mail: luigi.lucini@unicatt.it. Tel: +390523599156. Fax: +390523599358.

Table 1. The test microorganisms.

Microorganism	Gram (+/-)	Acting and importance
<i>E. coli</i>	-	Urinary tract infections
<i>B. cereus</i>	+	Different food contaminations, toxinogenic, production of tissue destructive exoenzymes
<i>B. licheniformis</i>	+	Food contaminations, peritonitis, septicaemia, gastroenteritis and bovine abortions
<i>S. epidermidis</i>	+	Commensally microorganism, human eyes diseases
<i>S. boulardii</i>	-	Beneficial intestinal effects and preventing diarrhoea

against *Staphylococcus aureus* strains and against *Escherichia coli*, probably through inhibition of solute transport in membranes (Hamman, 2008; Lone et al., 2009). The activity of *A. barbadensis* inner gel was reported as active against both gram positive and gram negative bacteria through several different approaches to determine the antimicrobial activity (Hamman, 2008; Habeeb et al., 2007). The antimicrobial properties of the *Aloe* gel have been ascribed to phenolic compounds which are present at low concentrations (Pawar et al., 2005), even if reported that it may result from the synergic activity of different compounds having a broad spectrum of antimicrobial activity (Lawrence et al., 2009).

A. barbadensis leaf gel can inhibit the growth of the gram negative bacteria *Shigella flexneri* and *Streptococcus pyogenes* (Ferro et al., 2003); it has been proposed that anthraquinones and dihydroxyanthraquinones have direct antimicrobial activity (Wu et al., 2006). Other studies have reported on the effect of the anthraquinone aloe emodin on arylamine N-acetyl transferase activity in *Helicobacter pylori* and hence its antimicrobial activity (Wang et al., 1998). *A. barbadensis* juice and gel are known to contain aloe emodin which is reported to be effective against several gram positive bacteria but was ineffective against a strain of *S. aureus* (Wu et al., 2006; Cock, 2008). Acemannans have been suggested to have indirect antimicrobial activity too, because of their ability to stimulate phagocytic leukocytes (Pugh et al., 2001). *Aloe* anthraquinone aloin is incapable of inhibiting the growth of some microbial agents while the inner gel was described as effective especially against gram negative bacteria (Cock, 2008) and against resistant strains of *Aspergillus niger* (Ferro et al., 2003).

As far as the plant material preparation is concerned, the maximum antibacterial activities were observed in the acetone extract rather than in the aqueous and ethanol extract (Arunkumar and Muthuselvam, 2009), although these agents extract only small quantities of anthraquinones. The object of this work was to assess the antimicrobial potential of different leaf fractions from the two main *Aloe* species (*A. barbadensis* Miller and *A. arborescens* Miller), and to compare this potential with the activity of pure anthraquinones. To test for the antimicrobial effects, four microorganisms, which are

pathogens for humans and animals were chosen, together with a beneficial microorganism (Table 1). Therefore, the experimental design is aimed to clarify the role of the main *Aloe* leaf fractions (and hence the different bioactive substances) in antimicrobial properties. In fact, comparative information about the contribution of each leaf fraction and therefore of anthraquinones and acemannans are not reported in literature yet, albeit they could help to explain the discrepancies reported by different authors.

MATERIALS AND METHODS

Plant material

The plants of *A. barbadensis* Mill. and *A. arborescens* Mill, three years old, were kindly supplied by Dester Gardens, Brescia, Italy. Considering the plant morphology and the different chemical composition of various parts, three fresh leaves of *A. barbadensis* from a basal, central and apical position were taken. As for *A. arborescens*, however, three fresh leaves from the central and three fresh leaves from the lateral stem were taken. The leaves of each plant were pooled and homogenized by a blender to prepare a representative sample. A further sampling, with the same scheme, was done to prepare leaf skin and pulp samples. With this purpose, leaves were cut and the outer green rind was separated from the inner parenchyma; each sample was minced and thoroughly homogenized by a blender.

Preparation of the test fractions

In our experiments, to improve the extraction of anthraquinones and acemannan, respectively, a methanol and ethyl-acetate mixture was used to extract plant derma whilst water was used to extract the inner leaf parenchyma. According to previous results, these solutions were identified as the most effective in both cases (data not shown). Hence, different extracts, gained from raw leaves of the two *Aloe* species considered, together with anthraquinone reference substances (aloin and aloe emodin, from Sigma Aldrich Chemical Company St. Louis, MO, USA) were tested. The green rind extracts were prepared by homogenizing 2 g of plant material in 4 + 4 ml of ethyl acetate/methanol (9:1 v/v), and the solids were separated by centrifugation (313 g for 15 min). The parenchyma extracts were prepared from 40 g of leaf pulp in 100 ml of double distilled sterile water, thoroughly homogenised and then shaken for 1.5 h. The pure aloin and aloe emodin solutions, however, were prepared by weighing an accurate amount of reference standard and diluting to volume with a methanol / water (1:1 v/v) mixture. Solutions were prepared with a concentration of 514 and 521 mg l⁻¹

for aloin and aloe emodin, respectively. All the samples were kept at +4°C, in a dark flask, until use.

Determination of anthraquinones and acemannan content

Anthraquinones were determined in each sample by liquid chromatography followed by tandem mass spectrometry with electrospray ionisation source (LC-ESI/MS/MS) in the negative mode and quantified by the external standard method. A 1200 series liquid chromatograph system was used, equipped with quaternary pump, electrospray ionization system and coupled to a G6410A triple quadrupole mass spectrometer detector (all from Agilent technologies, Santa Clara, CA, USA). The plant material (2 g) was extracted by Ultra-Turrax in 8 + 4 ml of ethyl acetate / methanol mixture (9:1 v/v) after adding 4 ml of 200 g l⁻¹ NaCl aqueous solution. After centrifugation (2000 g for 15 min) the extract was diluted with methanol, filtered through a 0.45 µm membrane and then analysed by reversed phase LC-MS/MS using a Zorbax Eclipse plus C18 column (10 mm length, 3 mm i.d., 3.5 µm film thickness) from Agilent. The solvent system used water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 ml min⁻¹; the gradient was designed to decrease solvent A from 35% at 0 min to 20% at 2.5 min.

The injection volume was 10 µl and the drying gas was nitrogen at 5 l min⁻¹. Data handling was performed by ChemStation software under Multiple Reaction Monitoring (MRM) acquisition: aloin transition was from m/z 417 (M-H)⁻ to 297 (collision energy 15 V), while aloe emodin transition was from m/z 269 (M-H)⁻ to 240 (collision energy 25 V). Furthermore polysaccharides were determined colorimetrically at 540 nm, after binding with Congo red dye, on the basis of the work described by different authors (Eberendu et al., 2005). Colorimetric measurements were done using a Perkin Elmer Ultraviolet/Visible (UV/VIS) spectrometer lambda 12. Aloe pulp (4 g) was extracted in 10 ml of double distilled water, on a horizontal shaker for 2 h; 500 µl of 15 g l⁻¹ KOH aqueous solution was added to the solution and then 2 ml of Congo red solution (obtained diluting a saturated aqueous solution 50 times) was also added. The solution was left for 1 h and then analysed at λ = 540 nm. Semi-quantitative determination of the solutions was carried out using a pure β-glucan standard. Three measurements on three different extracts were performed for each leaf pulp sample.

Microorganisms culture

Cell cultures of *E. coli*, *Bacillus cereus*, *Bacillus licheniformis*, *Staphylococcus epidermidis* known to cause health problems to humans and animals and *Saccharomyces boulardii*, a beneficial microorganism to human were chosen (Table 1) (Guslandi et al., 2000). The cultures of *E. coli*, *B. cereus* and *B. licheniformis*, *S. boulardii* and *S. epidermidis* were prepared in liquid broth 24 h before the inoculation. One sample of the culture taken by a bacteriological loop was put into 50 ml of sterile liquid broth and incubated at 30°C for *B. cereus* and *B. licheniformis*, and at 37°C for *E. coli*, *S. boulardii* and *S. epidermidis*.

Composition of the media

E. coli, *B. cereus* and *B. licheniformis* were incubated on Plate Count Agar medium (PCA, Biokar Diagnostics, France), for the aerobic plate count by the superficial streaking method composed of casein pectone (5 g l⁻¹), yeast extract (2.5 g l⁻¹), dextrose (1.0 g l⁻¹) and agar (15.0 g l⁻¹). It had a final pH of 7.0± 0.2; *S. epidermidis* on PCA composed of yeast extract (1 g l⁻¹), glucose (4 g l⁻¹),

chloramphenicol (0.02 g l⁻¹) and distilled water (200 ml); *S. boulardii* on CHC agar (Biokar Diagnostics, France) composed of yeast extract (1 g l⁻¹), glucose (4 g l⁻¹), chloramphenicol (0.02 g l⁻¹) and 200 ml of distilled water (pH 6.6).

Inoculation of the microorganisms and incubation

A volume of 0.3 ml of each microorganism culture suspension was put on the surface of the set agar in single-use plastic Petri dishes and left to absorb onto the culture. Then, three filter paper discs; 10 mm in diameter, impregnated with eight different solutions were put on the medium in the Petri dishes. For each microorganism and plant fraction, four replications were done, resulting in 128 Petri dishes in total. All Petri dishes were incubated in thermostat for three days at an appropriate temperature (*B. cereus* and *B. licheniformis* at 30°C, *E. coli*, *S. boulardii* and *Staph. epidermidis* at 37°C). The inhibition zones were measured in mm after 24, 48 and 72 h from inoculation.

Data analysis

The raw data were analyzed separately for each individual microorganism by one way ANOVA for the factor plant fraction with consequent testing of minimal significant differences using the Tukey HSD test (level of significance alpha = 0.05) in STATISTICA CZ 8.0. No difference among the plant fraction effects was assumed as the null hypothesis. The dynamics of the microorganism growth was shown as graph in Figure 1 and the final results (after 72 h of exposition) were chosen for the evaluation.

RESULTS AND DISCUSSION

Analysis of plant materials

Three different plants of the same age were extracted in triplicate and then analysed in duplicate. The results of LC-MS/MS and colorimetric analyses revealed that *A. barbadensis* whole leaf contained 1936±409 mg kg⁻¹ of aloin and 3767±752 mg kg⁻¹ of β-glucan equivalents while *A. arborescens* contained 1568±69 mg kg⁻¹ of aloin and 2242±88 mg kg⁻¹ of β-glucan equivalents, respectively. Aloe emodin could not be detected in both the species. Furthermore, the difference in aloin content between the two species was significant as shown by the t-test analysis (two tails, alpha = 0.01). The anthraquinone aloin (measured as the sum of the two stereoisomers aloin A and B) was substantially located in the outer green rind rather than in the inner parenchyma, as expected. Taking into account the different plant portions, the aloin content in *A. barbadensis* green rind was 1801 mg kg⁻¹, while it was 136 mg kg⁻¹ in gel; however *A. arborescens* contained 1286 mg kg⁻¹ of aloin in the green rind and 282 mg kg⁻¹ in the inner parenchyma.

Antimicrobial effect

The analysis of results (Table 2), shows that the effect of

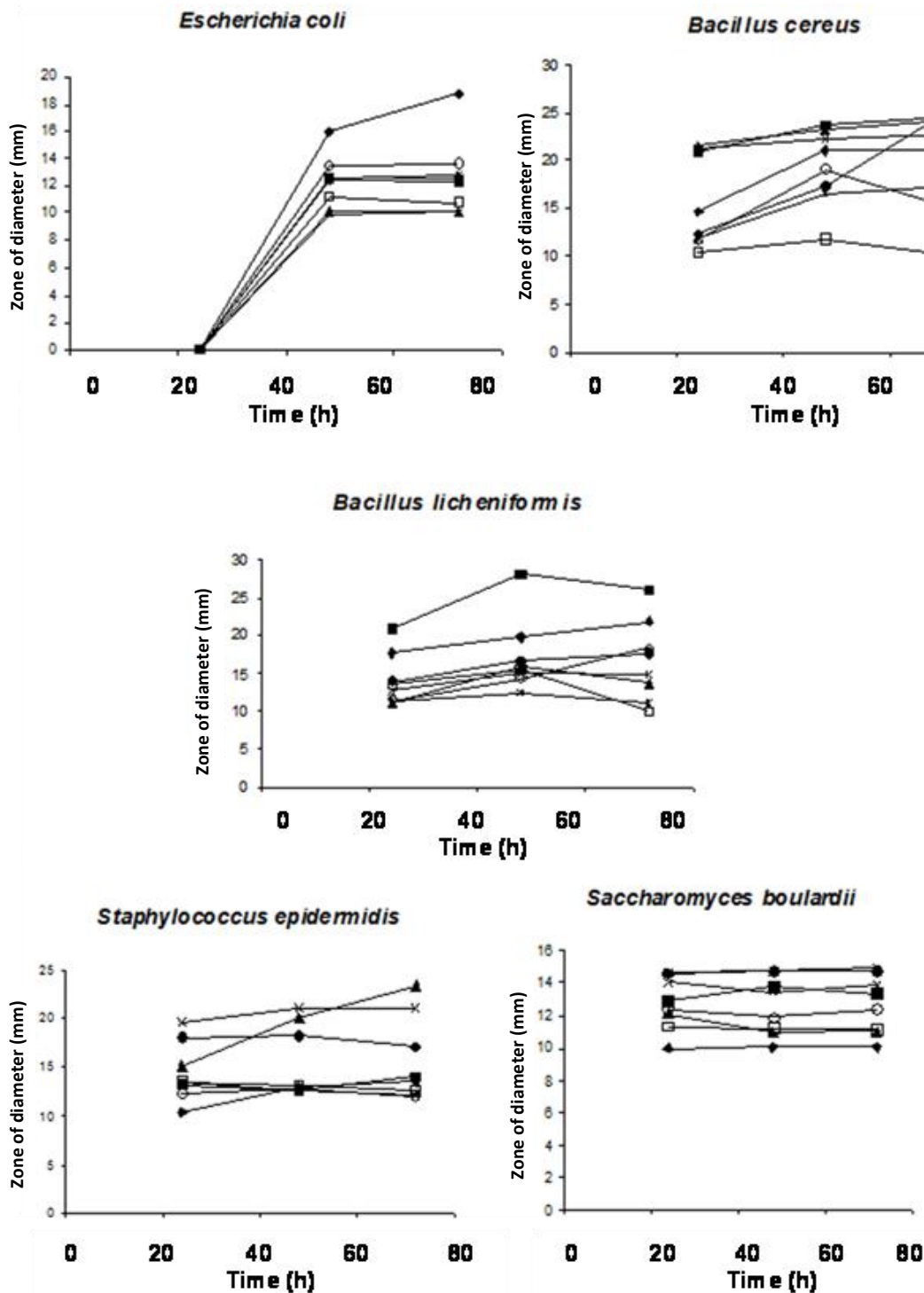


Figure 1. Dynamics of antibacterial activity of *Aloe* fractions: (◆) aloin, (■) aloe emodin, (▲) *A. barbadensis* gel, (X) *A. arborescens* gel, (*) *A. barbadensis* epidermis extract, (●) *A. arborescens* epidermis extract, (□) control 1 (methanol: water, 1:1), (○) control 2 (methanol: ethyl acetate, 9:1).

various *Aloe* fractions was statistically highly significant for all tested microorganisms after 72 h of incubation

(alpha = 0.01). The results were in good agreement: repeatability was in the range of 2.9 to 34.5% (in only

Table 2. Mean values of the inhibition zones diameter (mm, including the disc diameter) for different leaf fractions and pure compounds on microorganisms after 72 h.

Extract	Inhibition zone diameter (mm) ^a				
	<i>E. coli</i>	<i>B. cereus</i>	<i>B. licheniformis</i>	<i>S. epidermidis</i>	<i>S. boulardii</i>
Aloin standard solution	12.58±1.83 ^{ABC}	21.08±5.52 ^D	21.67±4.92 ^E	13.58±4.68 ^{AB}	10.08±0.29 ^A
Aloe emodin standard solution	12.33±2.39 ^{ABC}	24.42±2.23 ^D	25.91±2.02 ^F	14.00±3.30 ^{AB}	12.33±1.87 ^C
<i>Aloe barbadensis</i> gel (water extract)	10.17±0.58 ^A	24.17±1.53 ^D	13.67±1.97 ^{ABC}	23.33±3.89 ^D	11.08±1.38 ^{AB}
<i>Aloe arborescens</i> gel (water extract)	10.08±0.29 ^A	22.75±2.18 ^D	14.83±2.89 ^{BCD}	21.00±5.69 ^{CD}	13.92±1.73 ^C
Extract of <i>aloe barbadensis</i> epidermis	12.75±1.77 ^{BC}	17.25±5.82 ^{BC}	11.00±0 ^{AB}	12.25±1.84 ^A	14.92±3.70 ^C
Extract of <i>Aloe arborescens</i> epidermis	18.75±3.96 ^D	25.17±5.67 ^D	17.50±4.60 ^{CDE}	17.08±3.37 ^{BC}	14.7±2.70 ^C
Control 1 (methanol-water, 1:1)	10.75±1.14 ^{AB}	10.00±0 ^A	10.00±0 ^A	12.67±1.77 ^{AB}	11.17±1.40 ^{AB}
Control 2 (methanol-ethyl acetate, 1:9)	13.67±1.44 ^C	15.17±0.94 ^B	18.08±3.78 ^{DE}	12.00±1.21 ^A	12.33±2.19 ^{ABC}

^a Superscript uppercase letters denote statistically different groups.

one case). On this basis, the H_0 hypothesis (no difference in antimicrobial activity among the Aloe fractions) was rejected. The pure aloin standard solution was the most effective against *B. licheniformis*, while *S. boulardii* was the least affected microorganism. Pure aloe emodin standard solution reduced the growth of *B. licheniformis*, while the most resistant microorganisms were *E. coli* and *S. boulardii*. Hence, pure solutions of both substances were more effective against gram positive rather than the gram negative microorganisms. The water extracts of *A. barbadensis* and *A. arborescens* gel were the most effective against *B. cereus*, less effective against *E. coli*; between the two gels, the one from *A. barbadensis* showed a slightly stronger effect. Again, the gram negative microorganism (*E. coli*) and yeast *S. boulardii* were more resistant to these fractions in agreement with Lawrence et al. (2009).

Extract of *A. barbadensis* epidermis showed the strongest effect against *B. cereus* and the lowest against *B. licheniformis*. However, epidermis extract of *A. arborescens* showed the highest activity against *B. cereus* and the lowest activity against *S. boulardii*. In accordance with the data from the gel fraction, *A. arborescens* extract had a stronger effect rather than that of *A. barbadensis*. *E. coli* and *S. boulardii* were the most resistant microorganisms. Control variant 1 (methanol/water mixture) showed minimal activity against the microorganisms in comparison to the activity of the gel from both species solved in this solvent. The second control variant, the methanol/ethyl acetate mixture, showed relatively similar antibacterial activity to the epidermis extracts from both Aloe species, even though the extracts were more active. The trend of antimicrobial activity versus time was significantly different among the replicates. The standard solution of aloin showed a middle or lower effect against all microorganisms and, except *S. boulardii* which was not sensitive for the whole test duration, the antibacterial activity had an increasing effect.

The standard solution of aloe emodin had a stronger effect than the previous and showed increasing activity until 48 h; then, until 72 h, the activity against *B. licheniformis* and *S. boulardii* decreased. *S. epidermidis* was slightly reduced by aloe emodin until 24 h, after which the activity of the substance decreased, whereas it increased again after 72 h. Both fractions of the inner leaf portion showed relatively a high antibacterial effect, as compared to the two pure anthraquinone solutions, with the exception of *B. licheniformis* which was strongly sensitive to both aloin and aloe emodin. The gel fractions had similar activity against all microorganisms, with an increasing course in all cases. Generally, the gel from *A. arborescens* had a slightly stronger effect against all the microbial species tested, with the exception of *S. epidermidis*. The green rind extracts of both Aloe species also showed similar trend in time; an increasing effect was observed, again excepting for *S. epidermidis*. The course for *Staphylococcus* showed decreased activity from 24 to 72 h from the inoculation. The control variants represented by the solvents also showed some antibacterial activity against most of the microorganisms, comparable to those of *A. barbadensis* gel extract and pure aloin.

These control variants, however, had no activity against *B. cereus* and *B. licheniformis*. The effect of the fractions against *E. coli* started in the first 48 h, after that, the effect was steady. None of the fractions showed an effect against *S. boulardii* throughout the whole time period. The most resistant microorganisms were *E. coli* and *S. boulardii*. The gram negative microorganism (*E. coli*) was more resistant to all the tested fractions; similar results were achieved by other authors (Lawrence et al., 2009), although they assessed the activity of ethanol, methanol and acetone extracts of whole leaves. This resistance was possibly due to the presence of another lipopolysaccharide layer. On the other hand, gram positive microorganisms are more susceptible because of absence of this lipid layer, even the glycopeptidic layer is

thick (Jai et al., 2005). According to Arunkumar and Muthuselvam (2009), the most effective antibacterial fraction of whole leaves was the acetone extract rather than the ethanolic one, although Lawrence et al. (2009) asserted the contrary. In our experiments, methanol was the most effective solvent because of the largest inhibition zones, probably due to the wider extraction profile of the antimicrobial constituents from Aloe whole leaves (Mbangi et al., 2010). According to our results, the two Aloe species contain different amount of aloin in both epidermis and gel.

Generally, *A. barbadensis* contained a higher amount of both aloin and acemannan than *A. arborescens*. The antibacterial activity against the individual microorganisms was similar between *A. barbadensis* and *A. arborescens* gel, while the methanol/ethyl acetate extracts from the epidermis have shown higher activity for *A. arborescens*, despite its having lower amounts of aloin. Regarding the two controls, methanol is relatively very volatile and evaporates during the experiments, resulting in a lower interference. Ethyl acetate, however, is less volatile and is able to change the pH of the environment inside the cells, therefore acting as an antimicrobial agent. There are many discrepancies among authors about the antimicrobial components in Aloe plants, but in general it is clear that a synergistic effect of several compounds is likely to be the best explanation of this biological effect. Actually, anthraquinones are reported to act together with acemannans *in vivo*, but *in vitro* only the anthraquinones are effective; some studies on the oral administration of whole leaves of *A. barbadensis* reported that both substances work simultaneously and synergistically (Pandey and Mishra, 2010).

Some of the compounds to which the maximum antibacterial activity of *A. barbadensis* gel could be ascribed: *p*-coumaric acid, ascorbic acid, pyrocatechol, cinnamic acid (Lawrence et al., 2009). Recently some important constituents of *A. arborescens* leaves were identified, namely aloe emodin, feruloylaloenin, aloins A and B, aloenin, malic, malonic and oxalic acids and a dimethylchromone (Olennikov et al., 2009). Some of these compounds have a non polar character, and their relative toxicity to microorganisms was related to the site and number of hydroxyl groups on the phenol group. Our results confirmed that anthraquinones and aloe emodin in particular, can have an antimicrobial activity; similar conclusions were reported by Cock (2008). The leaf inner gel was antimicrobial as well and again some confirmation can be found in literature (Habeeb et al., 2007; Cock, 2008). The properties of the inner leaf gel were quite extensively faced; however the leaf outer green rind seems to be more interesting for antimicrobial solutions. Although the activity is strongly related to the microorganism considered, it is clear that different Aloe compounds, besides anthraquinones and acemannan,

are antimicrobial. These compounds probably act synergistically and with different mode of action. Furthermore, different Aloe species could have a different secondary metabolite profile, hence resulting in different antimicrobial properties. The present study shows the significant differences between the antimicrobial effect of individual fractions from the gel and epidermis of two species, *A. barbadensis* and *A. arborescens* comparing to the standard solutions of main anthraquinones. Although the effect of pure aloin and aloe emodin substances was confirmed, the activity of the methanol/ethyl acetate extracts from epidermis and those of the water extracts from the gel were higher. As discussed, a synergistic effect of several compounds is supposed and the plant species and leaf portion considered can play a major role in the definition of Aloe antimicrobial properties.

ACKNOWLEDGEMENTS

This study was supported by the Doctoral School on the Agro-Food System (Agrisystem) of the Università Cattolica del Sacro Cuore (Italy).

REFERENCES

- Arunkumar S, Muthuselvam M (2009). Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World J. Agric. Sci.*, 5: 572-576.
- Boudreau MD, Beland FA (2006). An evaluation of the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe vera*. *J. Environ. Sci. Health C*, 24: 103-154.
- Cock IE (2008). Antimicrobial activity of *Aloe barbadensis* Miller leaf gel components. *Internet J. Microb.*, 4(2).
- Eberendu AR, Luta G, Edwards A, McAnalley BH, Davis B (2005). Quantitative colorimetric analysis of *Aloe* polysaccharides as a measure of *Aloe vera* quality in commercial products. *J. Assoc. Off. Anal. Chem. int.*, 88: 684-691.
- Eshun K, He Q (2004). *Aloe vera*: A valuable ingredient for the food, pharmaceutical and cosmetic industries. *Food Sci. Nutr.*, 44: 91-96.
- Ferro VA, Bradbury P, Cameron E, Shaker SR, Stimson WH (2003). *In vitro* susceptibilities of *Shigella flexneri* and *Streptococcus phyogenes* to inner gel of *Aloe barbadensis* Miller. *Antimicrob. Agents Chemother.*, 47: 1137-1139.
- Guslandi M, Mezzi G, Sorghi M, Testoni PA (2000). *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. *Dig. Dis. Sci.*, 45: 1462-1464.
- Habeeb F, Shakir E, Bradbury F, Cameron P, Taravati MR, Drummond AJ, Gray AI, Ferro VA (2007). Screening methods used to determine the anti-microbial properties of *Aloe vera* inner gel. *Methods*, 42: 315-320.
- Hamman JH (2008). Composition and applications of *Aloe vera* leaf gel. *Molecules*, 13: 1599-1616.
- Lawrence R, Priyanka T, Ebenezer J (2009). Isolation, purification and evaluation of antibacterial agents from *Aloe vera*. *Bra. J. Microbiol.*, 40: 906-915.
- Liao HM, Sheng XY, Hu ZH (2006). Ultrastructural studies on the process of aloin production and accumulation in *Aloe arborescens* (*Asphodelaceae*) leaves. *Bot. J. Linn. Soc.*, 150: 241-247.
- Lone MA, Dinisha M, Pooja M, Aarti D, Safena RC (2009). Anti-inflammatory and antimicrobial activity of anthraquinone isolated from *Aloe vera* (*Liliaceae*). *Chem. Asian. J.*, 21: 1807-1811.

- Mbanga J, Mangoma N, Saidi B (2010). An evaluation of the antimicrobial activities of *Aloe barbadensis*, *A. chabaudii* and *A. arborescens* leaf extracts used in folklore veterinary medicine in Zimbabwe. *J. Anim. Vet. Adv.*, 23: 2918-2923.
- Moody JO, Adebisi OA, Adeniyi BA (2004). *Aloe vera* and *Ageratum conyzoides* enhance the anti-microbial activity of traditional medicinal soft soaps. *J. Ethnopharmacol.*, 92: 57-60.
- Olennikov DN, Ibragimov TA, Chelombiko VA, Nazarova AV, Rohkin AV, Zilfikarov IN (2009). Chemical composition of *Aloe arborescens* and its change by biostimulation. *Chem. Nat. Comp.*, 45: 478-482.
- Pandey R, Mishra A (2010). Antibacterial activities of crude extract of *Aloe barbadensis* to clinically isolated bacterial pathogens. *Appl. Biochem. Biotechnol.*, 160: 1356-1361.
- Pawar VC, Bagatharia SB, Thaker VS (2005). Antibacterial activity of *Aloe vera* leaf gel extracts against *Staphylococcus aureus*. *Indian J. Microbiol.*, 45: 227-229.
- Pugh N, Ross SA, Elsohly MA, Pasco DS (2001). Characterisation of aloeride, a new high molecular weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. *J. Agric. Food Chem.*, 49: 1030-1034.
- Rajurkar SR, Vadlamudi VP, Kulkarni DD, Qureshi MI (2001). *In vitro* antibacterial evaluation of *Aloe vera* leaf extracts. *J. Vet. Pharm. Toxicol.*, 1: 92-94.
- Wang HH, Chung JG, Ho CC, Wu CT, Chang SH (1998). Aloe-emodin effects on arylamine N-acetyl transferase activity in the bacteria *Helicobacter pylori*. *Planta Med.*, 64: 176-178.
- Wu YW, Ouyang J, Xiao XH, Gao WY, Liu Y (2006). Antimicrobial properties and toxicity of anthraquinones by microcalorimetric bioassay. *Chin. J. Chem. Eng.*, 24: 45-50.