

ORIGINAL ARTICLE

## ***In vitro* activity of *Aloe vera* inner gel against *Helicobacter pylori* strains**

L. Cellini, S. Di Bartolomeo, E. Di Campli, S. Genovese, M. Locatelli and M. Di Giulio

Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy

**Significance and Impact of the Study:** The study demonstrates that the *Aloe vera* inner gel expresses antibacterial properties against both susceptible and resistant *Helicobacter pylori* strains. These findings may impact on the antimicrobial resistance phenomenon of *H. pylori*, proposing the *A. vera* inner gel as a novel effective natural agent for combination with antibiotics for the treatment of *H. pylori* gastric infection.

### Keywords

*Aloe vera*, antibacterial activity, *Helicobacter pylori*, resistant strains, therapeutic regimens.

### Correspondence

Mara Di Giulio, Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, Via dei Vestini, 31, Chieti, Italy.  
E-mail: m.digiulio@unich.it

2013/2419: received 3 December 2013, revised 17 February 2014 and accepted 28 February 2014

doi:10.1111/lam.12241

### Abstract

*Aloe barbadensis* Miller (*Aloe vera*) is a herbal remedy widely used for a variety of illnesses; *A. vera* leaf extracts have been promoted for detoxification, cure constipation, help flush out toxins and wastes from the body, promote digestion and are used in the treatment of peptic ulcer for cytoprotective action. The aim of this study was to evaluate the antibacterial activity of *A. vera* inner gel against both susceptible and resistant *Helicobacter pylori* strains isolated in Abruzzo region, Italy. The inner gel of leaves of a 5-year-old plant of *A. vera* was extracted, homogenized and tested from 800 to 1.56 mg ml<sup>-1</sup> against 14 clinical strains and one reference strain of *H. pylori* using the broth microdilution methodology. Furthermore, the sample of *A. vera* was investigated for the chemical fingerprint of anthraquinones. The inhibitory concentrations of *A. vera* inner gel were similar to the bactericidal ones, with values ranging from 6.25 to 800 mg ml<sup>-1</sup>. Fifty per cent of the detected strains, independently of their susceptibility profile, were inhibited in their growth at 100 mg ml<sup>-1</sup>. *Aloe vera* inner gel expresses antibacterial properties against *H. pylori* and, therefore, in combination with antibiotics, could represent a novel strategy for the treatment of the infection of *H. pylori*, especially in cases of multiresistance.

### Introduction

*Helicobacter pylori* is a Gram-negative bacterium that colonizes the human stomach early in life, although the related pathology may be expressed later. Half of the people worldwide are carriers of this micro-organism, but disease occurs in only about 15%, with the development of gastritis, peptic ulcer, gastric adenocarcinoma and MALT lymphoma (Bhandari and Crowe 2012).

Eradication treatments have been developed during the last 20 years, leading to a decrease in *H. pylori*-related peptic ulcer disease and in the prevalence of the infection in the Western world (Tepes *et al.* 2012). However, the success of these treatments is recently compromised by

the development of antimicrobial resistance (Megraud *et al.* 2013). In fact, the antibiotic resistance is the key factor for the treatment failure (Suzuki *et al.* 2010). In particular, the increasing resistance to clarithromycin and quinolones compromises the eradication rate significantly, thus suggesting, before treatment, to monitor the *H. pylori*-resistant profile improving its eradication (Marzio *et al.* 2011). The emergence of resistant strains, often due to overuse or misuse of common antibiotics, overshadows the therapeutic effect of traditional treatments, and alternative regimes have been proposed (Bisignano *et al.* 2013).

Medicinal plants, herbs and fruit extracts have also been shown to possess antimicrobial activity against

*H. pylori* (Cellini *et al.* 1996; Tabak *et al.* 1999; Nostro *et al.* 2005, 2006; Pattiyathane *et al.* 2009; Vega *et al.* 2009; Bisignano *et al.* 2013), and their valuable effect could be considered as an important alternative or adjuvant therapeutic approach. Awareness is now growing regarding the preferred use of medicinal plant materials as prophylaxis and therapeutics over the synthetic drugs.

Some plants with well-recognized abundant anthraquinone profile such as *Rhamnus* and *Frangula* species show antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* (Locatelli *et al.* 2011; Kremer *et al.* 2012).

*Aloe vera*, a traditional Chinese medicine, which includes anthraquinones among its active components, has been used as a curative agent for the treatment of a multitude of diseases.

*Aloe vera* inner gel is said to promote wound healing due to the presence of components such as anthraquinones, which possess antibacterial, antifungal and antiviral activities. It has a well-established antimicrobial activity ascribed to compounds that are now specifically identified as p-coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid (Lawrence *et al.* 2009).

*Aloe vera* was found to have extraordinary anti-ulcerogenic effects, inhibiting gastric acid secretion and gastric lesion, protecting from mucosal injury and accelerating the cicatrization of gastric ulcers (Foster *et al.* 2011).

*In vitro* studies have shown that *A. vera* inner gel displayed an antimicrobial effect against both Gram-negative and Gram-positive isolates (Habeeb *et al.* 2007; Bashir *et al.* 2011).

Hence, the main goal of the present study was to search for the presence of antimicrobial activity of *A. vera* inner gel against susceptible and resistant *H. pylori* strains and also to determine the chemical fingerprint of major anthraquinones of the *A. vera* inner gel used.

## Results and discussion

From a clinical standpoint, the prospect of a novel, therapeutic measure aimed at improving the eradication of *H. pylori* is of special importance, and this is also matched by a renewed interest in the anti-infective properties of medicinal plants and foods.

The qualitative and quantitative analyses of the *A. vera* inner gel are reported in Table 1. *Aloe-emodin* and *chrysophanol* resulted to be the most abundant compounds with values of 39.2 and 30.5%, respectively. The other three 'classic' anthraquinones were found to be present in less quantity (from 1/2 to 1/3).

In Table 2 shown is the antimicrobial activity of *A. vera* inner gel against *H. pylori* strains paralleled to the

**Table 1** Qualitative and quantitative analyses of *Aloe vera* inner gel samples

	Retention time (min)	Concentration (mg ml <sup>-1</sup> )	Relative abundances (%)
<i>Aloe-emodin</i>	8.50	10.02	39.2
<i>Rhein</i>	12.7	1.31	5.1
<i>Emodin</i>	22.7	2.25	8.8
<i>Chrysophanol</i>	24.6	7.80	30.5
<i>Physcion</i>	27.7	4.18	16.4
Total		25.56	

**Table 2** *In vitro* antibacterial activity (minimum inhibitory concentration, MIC; and minimum bactericidal concentration, MBC) of *Aloe vera* inner gel and amoxicillin against *Helicobacter pylori* strains

<i>H. pylori</i> strains	<i>Aloe vera</i> (MIC/MBC) mg ml <sup>-1</sup>	Amoxicillin (MIC/MBC) µg ml <sup>-1</sup>
ATCC 43629	100/100	0.06/0.12
15/2012/A	200/400	0.24/0.48
8/2012/A	200/200	0.12/0.48
6/2012/A	400/400	0.24/0.48
16/2012/A	25/50	0.06/0.12
10/2012/A	200/400	0.12/0.24
1/2013/A	200/400	0.12/0.12
3/2013/A	100/200	0.24/0.48
1/2012/A	800/800	0.06/0.06
2/2012/A	400/400	0.06/0.12
4/2012/A	100/200	0.06/0.12
8/2011/A	6.25/6.25	0.06/0.24
13/2011/A	400/800	0.24/0.48
21/2011/A	100/100	0.12/0.24
2/2010/A	400/400	0.24/0.48

AMX antimicrobial activity. The MIC values of *A. vera* inner gel ranged from 6.25 to 800 mg ml<sup>-1</sup>, with the growth of 50% of the detected strains being inhibited in the presence of 100 mg ml<sup>-1</sup> of *A. vera* inner gel, whereas 400 mg ml<sup>-1</sup> was required to inactivate 90% of them. Interestingly, the MIC values *A. vera* inner gel were similar to MBC values except for few cases in which only one step-up was recorded to obtain the bacterial death.

These data were of particular interest when compared to the *H. pylori* susceptibility panels against antimicrobial agents commonly used in therapy (Table 3). In fact, the detected *H. pylori* strains displayed multiresistant profiles with very high resistant rate to CLA and quinolones (53.3, 40.0, 26.7 and 20.0% for CLA, LE, MO and CIP, respectively). Moreover, the strains that presented a high percentage of antimicrobial resistance did not express similar behaviour against *A. vera* inner gel. In particular, *H. pylori* 3/2013/A, *H. pylori* 4/2012/A, *H. pylori* 8/2011/A and *H. pylori* 21/2011/A multiresistant strains were inhibited

**Table 3** Susceptibility patterns (resistant, R; and susceptible, S) of *Helicobacter pylori* strains against antimicrobial agents

<i>H. pylori</i> strains	Antimicrobial agents								
	CLA*	MET	LE	MOX	CIP	TET	AMX	RIF	AMP
ATCC 43629	S	S	S	S	S	S	S	S	S
15/2012/A	S	S	S	S	S	S	S	S	S
8/2012/A	S	S	S	S	S	S	S	S	S
6/2012/A	S	S	S	S	S	S	S	S	S
16/2012/A	S	S	S	S	S	S	S	S	S
10/2012/A	S	S	S	S	S	S	S	S	S
1/2013/A	S	S	R	R	S	S	S	S	S
3/2013/A	R	S	R	R	R	S	S	S	S
1/2012/A	R	R	S	S	S	S	S	S	S
2/2012/A	R	R	R	R	R	S	S	S	S
4/2012/A	R	S	R	S	S	S	S	S	S
8/2011/A	R	R	S	S	S	S	S	S	S
13/2011/A	R	S	S	S	S	S	S	S	S
21/2011/A	R	R	R	R	S	S	S	S	S
2/2010/A	R	S	R	S	R	S	S	S	S
Percentage of strains resistant to antimicrobial	53.3	26.7	40.0	26.7	20.0	0	0	0	0

\*see Materials and methods.

*in vitro* by *A. vera* inner gel at concentrations never greater than 100 mg ml<sup>-1</sup> (Table 2).

The anti-*H. pylori* properties of *A. vera* inner gel against multiresistant strains emphasize the need to find new natural therapeutic substances as adjuvant in the traditional therapies that should be proved in terms of effectiveness *in vivo*. Moreover, as suggested by Pandey and Mishra (2010), *A. vera* inner gel could be more effective when taken orally, because, *in vivo*, both acemannans and anthraquinones were capable of guaranteeing their full activity.

The increasing development of antimicrobial resistance in *H. pylori* strains is a worldwide concern, and the use of medicinal plants could be a potential benefit as anti-*H. pylori* agents in combined therapies with chemical agents for improving the eradication, in particular in cases of multidrug resistance. Several studies show additive and synergistic *in vitro* effects of plant extracts in combination with different antibiotics against *H. pylori* strains, supporting the idea that natural compounds could be a new strategy for developing therapies for microbial infections (Nostro *et al.* 2006; Lawal *et al.* 2012).

The inhibitory activity of *A. vera* against many microorganisms (Habeeb *et al.* 2007) has been mentioned in clinical reports for nearly 100 years, and its use can be particularly important when traditional therapies are found to be too inefficacious (Gupta and Malhotra 2012).

*Aloe vera* is widely utilized as a folk remedy for its antimicrobial and other beneficial effects. The inner gel composition is primarily water (99.0–99.5%); the remaining 0.5–1.0% is made up of solid material with more than 75

different potentially bioactive compounds, including vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds and organic acids (Atherton 1998). Phytochemically, polysaccharides have been characterized as the main component in *A. vera*, and they are responsible for many of its biological activities. In particular, carbohydrates play an important role in bacterial adhesion to the host, and some oligo-/polysaccharides have been reported to be effective for the inhibition of *H. pylori* adhesion (Xu *et al.* 2010). This anti-adhesive effect exhibited *in vitro* by *A. vera* on gastric cells together with the antimicrobial activity against *H. pylori* could represent an efficacious combination to counteract the bacterial infection. Recently, Ranade *et al.* (2012) developed an innovative floating tablet with *A. vera* in combination with amoxicillin to contrast the *H. pylori*-induced peptic ulcer, showing that *A. vera* could be a source of new co-therapeutics. In this innovative system, *A. vera* inner gel improves the amoxicillin adsorption through its retention in the gastric mucosa.

Further studies are also required to determine the active properties of numerous other *A. vera* constituents and to explore the competitive or synergistic actions of particular combinations of ingredients.

Being bacterial resistance a global problem of public health, and because the failure of *H. pylori* treatments has become more frequent lately, the discovery of new compounds with promising anti-*H. pylori* effect used in combination with antibiotics could represent a novel strategy for the treatment of gastric infection of *H. pylori*. More *in vitro* and *in vivo* studies should be conducted to confirm these results.

## Materials and methods

### Bacteria and preparation of inocula

Fourteen strains of *H. pylori* isolated from antral mucosal biopsies of patients with dyspeptic symptoms were used; the strains were identified on the basis of colony appearance, Gram staining and positive reactions in biochemical tests (catalase, urease and oxidase). All strains were stored at  $-80^{\circ}\text{C}$  for long-term maintenance (Cellini *et al.* 1996). Each strain was previously tested against clarithromycin (CLA), metronidazole (MET), levofloxacin (LE), moxifloxacin (MO), ciprofloxacin (CIP), tetracycline (TET), amoxicillin (AMX), rifabutin (RIF) and ampicillin (AMP) that are usually used in therapy; the strains isolated in 2012 were chosen on the basis of their susceptible and resistant profiles. Susceptibility testing was performed and interpreted according to a European study protocol (Megraud *et al.* 2013) in which some of the strains used in this work were included. For MO, CIP and AMP were used the breakpoints furnished by Cheng *et al.* (2012), Boyanova *et al.* (2008) and Falsafi *et al.* (2009), respectively.

*Helicobacter pylori* ATCC 43629 was used as a standard control strain. For the inoculum, strains were cultured in duplicate on nonselective medium (CA, chocolate agar) containing Columbia agar base (Oxoid, Milan, Italy) with 10% (v/v) laked horse blood plus IsoVitaleX 1% (v/v) (BBL; Microbiology System, Milan, Italy) and incubated in a microaerobic environment at  $37^{\circ}\text{C}$  for 5–7 days (GasPak, Oxoid). The bacterial suspensions, containing all colonies grown on CA medium, were prepared in Brucella Broth (BB) (Biolife Italiana, Milan, Italy) and adjusted to  $0.2$  ( $1.8 \times 10^7$  CFU  $\text{ml}^{-1}$ ) using Biophotometer (Eppendorf, Milan, Italy).

### Extraction of *Aloe vera* inner gel

The plants of *A. vera* var *barbadensis* Miller were retrieved at a local farmer plant in Manoppello, Abruzzo, Italy. The *A. vera* came from the Farm Francesco Amati (Contrada Ciarbo, 6, 72017 Ostuni, Italy) that manufactures and sells plants of *A. vera* and *arborescens*. The plants were grown without chemical fertilizers (on a fundus) in about an acre of land in the city of Ostuni ( $40^{\circ}44'00''$  South and  $17^{\circ}35'00''$  West) away from sources of pollution. On the farm, there were approx. 30 000 plants of all ages. Mature and fresh leaves of *A. vera*, of a 5-year-old plant, approx. 70–80 cm long were washed with fresh water, their thick epidermides were removed, and the inner gel was cut into pieces.

The solid, mucilaginous, thick, transparent gel was collected in a dark sterile container for light protection. The *A. vera* inner gel was homogenized with an Ultra-Turrax

(T 10 basic ULTRA-TURRAX) (IKA-Lab, Milan, Italy) in 2% dimethyl sulphoxide. To sterilize, the solution was boiled and filtered with  $0.80\text{-}\mu\text{m}$  filter (Millipore, SpA, Rome, Italy) and used immediately after. No chemicals were added, and the original composition was maintained.

### HPLC–UV/vis determination and chemical fingerprint of 5 anthraquinones in *Aloe vera* inner gel

Emodin (>99%), rheine (>99%), chrysophanol (>99%), *Aloe*-emodin (>99%) and physcion (>99%) were purchased from Extrasynthese (Genay, France). Methanol (HPLC grade) and formic acid (99%) were obtained from Carlo Erba Reagenti (Milan, Italy). Double-distilled water was obtained using a Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp., Bedford, MA).

HPLC analyses were performed on a Waters liquid chromatograph equipped with a model 600 solvent pump and a 2996 photodiode array detector, and the mobile phase was directly online degassed using Degassex, mod. DG-4400 (Phenomenex, Torrance, CA). Empower ver. 2 software (Waters Spa, Milford, MA) was used for data acquisition and elaboration. An ODS reverse-phase packing column (GraceSmart RP18,  $4.6\text{ m} \times 150\text{ mm}$ ,  $5\text{ }\mu\text{m}$ ; Grace, Deerfield, IL) was employed for the separation, and the column was thermostated at  $28 \pm 1$  using a Jetstream2 Plus column oven. For quantitative analyses, selective detection was performed at 435 nm. Gradient elution mode was performed using a mobile phase containing a 35 : 65 water–methanol ratio both with percentage of formic acid (v/v) as starting conditions and gradient program as reported in Table 4. All the sample solutions were previously centrifuged, and 20  $\mu\text{l}$  of the supernatant, after a filtration on Phenex-TFE (4 mm,  $0.45\text{ }\mu\text{m}$ ) syringe filters (Phenomenex, Torrance, CA), was injected into the HPLC–UV/vis system.

Combined working solutions of mixed standards at the concentrations of 0.5, 1, 5, 10, 25, 50, 75, 100 and  $125\text{ }\mu\text{mol l}^{-1}$  (corresponding to a linearity range from 0.127 to  $50.78\text{ }\mu\text{g ml}^{-1}$ , depending on analytes) were

**Table 4** Starting conditions and gradient program

Time (min)	Flow ( $\text{ml}^{-1}\text{ min}$ )	%A*	%B†
0		35	65
15		35	65
20		10	90
45	0.7	10	90
47		35	65
60		35	65

\*HPLC-grade water + 1% formic acid (v/v).

†Methanol + 1% formic acid (v/v).

obtained by dilution of a mixed stock solution at  $1 \text{ mmol l}^{-1}$  in volumetric flasks containing the mobile phase. Finally, the nine calibration standards were injected into the HPLC–UV/vis system. Calibration curves were calculated by analysing these nine nonzero concentration standards prepared in freshly spiked solution in triplicate. All quantitative analyses were performed at 435 nm. Calibration curves were plotted using weighted linear least-squares regression analysis according to the equation  $y = a + bx$ , where ‘y’ is the analyte peak area, ‘x’ represents the analyte concentration ( $\mu\text{mol l}^{-1}$ ) in the calibration samples, ‘a’ is the intercept and ‘b’ is the slope of the regression line. Concentrations of the QCs and unknown samples were calculated by interpolating their analyte peak area on the calibration curve.

#### Detection of minimum inhibitory concentration and minimum bactericidal concentration

The antibacterial activity of *A. vera* inner gel was assayed by broth microdilution method according to CLSI guidelines (2007). Serial dilutions of AMX (Sigma-Aldrich Co., St Louis, MO) were used as a control in the susceptibility test.

Twofold dilutions of substances were performed to obtain the following final concentrations: from 800 to  $1.56 \text{ mg ml}^{-1}$  for *A. vera* inner gel and from 500 to  $0.24 \mu\text{g ml}^{-1}$  for AMX. Broth microdilution methods were carried out in 96-well microtitre plates. In each well were dispensed aliquots ( $100 \mu\text{l}$ ) of each dilution of tested substances and aliquots ( $100 \mu\text{l}$ ) of each bacterial suspension adjusted to  $0.2 (1.8 \times 10^7 \text{ CFU ml}^{-1})$  using Biophotometer (Eppendorf, Milan, Italy), as described earlier.

Two hundred microlitres of substances, bacterial suspensions and BB were also included as controls. Plates were incubated in microaerobic condition at  $37^\circ\text{C}$  for 3 days. MIC was measured by determining the smallest amount of extract or antibiotic needed to inhibit the visible growth of the micro-organism. For the MBC, aliquots ( $5 \mu\text{l}$ ) of suspensions without visible growth were replicated per spot on CA plates and incubated at  $37^\circ\text{C}$  for 3–5 days under microaerobic conditions (Pattiyathanee *et al.* 2009). The MBC was determined as the lowest concentration of *A. vera* inner gel or AMX that gave complete inhibition of colony formation on CA plates (see graphical abstract).

Experiments were performed in triplicate.

#### Acknowledgements

We thank Mrs. Maria Grazia and Fulvia Gloria who furnished the *Aloe vera* plant used for the experiments carried out in this study, Dr. Mariana Alexandra Feliciano

Jorge for useful contributions to this study and Dr. Olivetta Del Bianco for English revision.

#### Conflict of Interest

The authors have no conflicts of interest to declare.

#### References

- Atherton, P. (1998) *Aloe vera*: magic or medicine? *Nurs Stand* **12**, 49–52.
- Bashir, A., Saeed, B., Talat, Y. and Jehan, M.N. (2011) Comparative study of antimicrobial activities of *Aloe vera* extracts and antibiotics against isolates from skin infections. *Afr J Biotechnol* **10**, 3835–3840.
- Bhandari, A. and Crowe, S.E. (2012) *Helicobacter pylori* in gastric malignancies. *Curr Gastroenterol Rep* **14**, 489–496.
- Bisignano, C., Filocamo, A., La Camera, E., Zummo, S., Fera, M.T. and Mandalari, G. (2013) Antibacterial activities of almond skins on *cagA*-positive and-negative clinical isolates of *Helicobacter pylori*. *BMC Microbiol* **13**, 103.
- Boyanova, L., Gergova, G., Nikolov, R., Davidkov, L., Kamburov, V., Jelevev, C. and Mitov, I. (2008) Prevalence and evolution of *Helicobacter pylori* resistance to 6 antibacterial agents over 12 years and correlation between susceptibility testing methods. *Diagn Microbiol Infect Dis* **60**, 409–415.
- Cellini, L., Di Campli, E., Masulli, M., Di Bartolomeo, S. and Allocati, N. (1996) Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). *FEMS Immunol Med Microbiol* **13**, 273–277.
- Cheng, A., Sheng, W.H., Liou, J.M., Wang, H.P., Wu, M.S., Lin, J.T. and Chang, S.C. (2012) Comparative *in vitro* antimicrobial susceptibility and synergistic activity of antimicrobial combinations against *Helicobacter pylori* isolates in Taiwan. *J Microbiol Immunol Infect.* pii: S1684-1182(12)00182-X. doi: 10.1016/j.jmii.2012.08.021. [Epub ahead of print].
- Clinical and Laboratory Standards Institute (2007) *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard, M11–A7 & M11–S*, 7th edn. Wayne, PA: Clinical and Laboratory Standards Institute.
- Falsafi, T., Ehsani, A. and Niknam, V. (2009) The role of active efflux in antibiotic-resistance of clinical isolates of *Helicobacter pylori*. *Ind J Med Microbiol* **27**, 335–340.
- Foster, M., Hunter, D. and Samman, S. (2011) Evaluation of the nutritional and metabolic effects of *Aloe vera* Chapter 3 in. In *Herbal Medicine: Biomol Clinic Aspects*, 2nd edn. ed. Benzie, I.F.F. and Wachtel-Galor, S. Boca Raton, FL: CRC Press.
- Gupta, V.K. and Malhotra, S. (2012) Pharmacological attribute of *Aloe vera*: revalidation through experimental and clinical studies. *Ayu* **33**, 193–196.

- Habeeb, F., Stables, G., Bradbury, F., Nong, S., Cameron, P., Plevin, R. and Ferro, V.A. (2007) The inner gel component of *Aloe vera* suppresses bacterial-induced pro-inflammatory cytokines from human immune cells. *Methods* **42**, 388–393.
- Kremer, D., Kosalec, I., Locatelli, M., Epifano, F., Genovese, S., Carlucci, G. and Končić, M.Z. (2012) Anthraquinone profiles, antioxidant and antimicrobial properties of *Frangula rupestris* (Scop.) Schur and *Frangula alnus* Mill. Bark. *Food Chem* **131**, 1174–1180.
- Lawal, T.O., Adeniyi, B.A., Moody, J.O. and Mahady, G.B. (2012) Combination studies of *Eucalyptus torelliana* F. Muell. leaf extracts and clarithromycin on *Helicobacter pylori*. *Phytother Res* **26**, 1393–1398.
- Lawrence, R., Tripathi, P. and Jayakumar, E. (2009) Isolation purification and evaluation of antibacterial agents from *Aloe vera*. *Braz J Microbiol* **40**, 906–915.
- Locatelli, M., Epifano, F., Genovese, S., Carlucci, G., Končić, M.Z., Kosalec, I. and Kremer, D. (2011) Anthraquinone profile, antioxidant and antimicrobial properties of bark extracts of *Rhamnus catharticus* and *R. orbiculatus*. *Nat Prod Commun* **6**, 1275–1280.
- Marzio, L., Cellini, L., Amitrano, M., Grande, R., Serio, M., Cappello, G. and Grossi, L. (2011) *Helicobacter pylori* isolates from proximal and distal stomach of patients never treated and already treated show genetic variability and discordant antibiotic resistance. *Eur J Gastroenterol Hepatol* **23**, 467–472.
- Megraud, F., Coenen, S., Versporten, A., Kist, M., Lopez-Brea, M., Hirschl, A.M., Andersen, L.P., Goossens, H. et al. (2013) *Helicobacter pylori* resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut* **62**, 34–42.
- Nostro, A., Cellini, L., Di Bartolomeo, S., Di Campli, E., Grande, R., Cannatelli, M.A., Marzio, L. and Alonzo, V. (2005) Antibacterial effect of plant extracts against *Helicobacter pylori*. *Phytother Res* **19**, 198–202.
- Nostro, A., Cellini, L., Di Bartolomeo, S., Cannatelli, M.A., Di Campli, E., Procopio, F., Grande, R., Marzio, L. et al. (2006) Effects of combining extracts (from propolis or *Zingiber officinale*) with clarithromycin on *Helicobacter pylori*. *Phytother Res* **20**, 187–190.
- Pandey, R. and Mishra, A. (2010) Antibacterial activities of crude extract of *Aloe barbadensis* to clinically isolated bacterial pathogens. *Appl Biochem Biotechnol* **160**, 1356–1361.
- Pattiyathane, P., Vilaichone, R. and Chaichanawongsaroj, N. (2009) Effect of curcumin on *Helicobacter pylori* biofilm formation. *Afr J Biotechnol* **8**, 5106–5115.
- Ranade, A.N., Wankhede, S.S., Ranpise, N.S. and Mundada, M.S. (2012) Development of bilayer floating tablet of amoxicillin and *Aloe vera* gel powder for treatment of gastric ulcers. *AAPS PharmSciTech* **13**, 1518–1523.
- Suzuki, H., Nishizawa, T. and Hibi, T. (2010) *Helicobacter pylori* eradication therapy. *Future Microbiol* **5**, 639–648.
- Tabak, M., Armonb, R. and Neeman, I. (1999) Cinnamon extracts' inhibitory effect on *Helicobacter pylori*. *J Ethnopharmacol* **67**, 269–277.
- Tepes, B., O'Connor, A., Gisbert, J.P. and O'Morain, C. (2012) Treatment of *Helicobacter pylori* infection 2012. *Helicobacter* **17**(Suppl 1), 36–42.
- Vega, A.E., Wendel, G.H., Maria, A.O. and Pelzer, L. (2009) Antimicrobial activity of *Artemisia douglasiana* and dehydroleucodine against *Helicobacter pylori*. *J Ethnopharmacol* **124**, 653–655.
- Xu, C., Ruan, X.M., Li, H.S., Guo, B.X., Ren, X.D., Shuang, J.L. and Zhang, Z. (2010) Anti-adhesive effect of an acidic polysaccharide from *Aloe vera* L. var. *chinensis* (Haw.) Berger on the binding of *Helicobacter pylori* to the MKN-45 cell line. *J Pharm Pharmacol* **62**, 1753–1759.