Polish Journal of Microbiology
2014, Vol. 63, No 1, 3–14

MINIREVIEW

Use of Plant Extracts to Control and Treat AB_5 Enterotoxin-Related Diarrhea

MAGDALENA KOMIAZYK[1, 2], MAŁGORZATA PALCZEWSKA[1], IZABELA SITKIEWICZ[3] and PATRICK GROVES[1]*

[1] Laboratory of Molecular Interactions and NMR, Instituto de Tecnologia Química e Biológica (ITQB-UNL), Oeiras, Portugal
[2] Laboratory of Biochemistry of Lipids, Nencki Institute of Experimental Biology, Warsaw, Poland
[3] Department of Epidemiology and Clinical Microbiology, National Medicines Institute, Warsaw, Poland

Submitted 27 December 2013, accepted 30 December 2013

Abstract

Plants contain a broad spectrum of small molecules with potential antimicrobial properties. Here, we review the antimicrobial activities of plant extracts against enterotoxic bacteria encoding AB_5 toxins, including *Vibrio cholerae*, *Shigella dysenteriae* and enterotoxic *Escherichia coli* strains. Several plant extracts have strong antimicrobial effects and the potential to boost Oral Rehydration Therapy, which is the first line of treatment for acute diarrhea.

Key words: *Escherichia coli*, *Shigella dysenteriae*, *Vibrio cholerae*, AB_5 enterotoxin, plant extracts

1. Introduction

The World Health Organization (WHO) prepares yearly reports on the global status of diarrheal diseases (http://www.who.int/topics/diarrhoea/en/). In addition to reporting statistics for the number of cases and fatalities, the WHO identifies actions to reduce the spread of disease and improve treatments. In developed, rich countries, diarrhea is usually associated with poor hygiene standards in the food industry but in poor countries the majority of cases are related to a lack of clean water supplies and underdeveloped medical networks.

Diarrheal symptoms can be caused by multiple inflammatory bowel diseases such as ulcerative colitis, Crohn's disease or irritable bowel syndrome. However, symptoms can also be caused by a wide range of bacteria (*Campylobacter* sp., *Clostridium* sp., *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella dysenteriae*, enterotoxic strains of *Escherichia coli*), viruses (rotaviruses, noroviruses) and parasites (*Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium* sp.). Because of the difficulties in diagnosis and the need of rapid therapy to prevent water and ion loss, rehydration therapy is used as a first line of action.

Oral Rehydration Therapy (ORT) provides a simple and rapid treatment that can be given by any adult to a suffering individual, either adult or a child, to improve recovery from diarrhea. ORT solutions are usually composed of water, sodium chloride, glucose, potassium ions and citrate and the formulation currently recommended by the WHO has an osmolarity of 245 mmol/L (Anonymous, 2002). In contrast, intravenous rehydration requires a nurse while drugs require a doctor and a significant period of time to start working. It must be stressed that ORT alleviates the symptoms and aids recovery but does not cure the source of the diarrheal episode. The treatment of diarrhea depends on the initial source, which often is not known. It has been suggested that the use of antibiotics for the treatment of diarrhea worsens the problem in cases related to infections with bacteria carrying phages encoding endotoxins (Prins, 1994). Therefore, ORT is of particular importance where the infectious agent has already been removed by the diarrheal episode.

Edible plants are traditionally used by many societies to alleviate and cure diarrhea. The properties of traditional plant extracts are worth exploring as they can stop or kill bacterial growth, neutralize or deactivate enterotoxins, are cheap and readily available, can give a better flavor to ORT, provide useful microelements and vitamins, and last, but not least, edible plants do not require the same level of extensive testing or

* Corresponding author: P. Groves, Molecular Interactions and NMR, Instituto de Tecnologia Química e Biológica (ITQB-UNL), Av. da República (Estação Agronómica Nacional), 2780-157 Oeiras Oeiras, Portugal; phone: (+351) 214 469 300; fax: (+351) 214 411 277; **e-mail: pgroves@itqb.unl.pt**

regulatory approval as new drugs. This review covers the scientific literature relevant to the use of plant extracts to treat enterotoxic bacterial disease, particularly diarrhea caused by the AB_5 family of enterotoxins (*V. cholera*, *S. dysenteriae* and enterotoxin producing strains of *E. coli*). Together, the organisms that produce AB_5 enterotoxins result in more than 200 million episodes of diarrhea per year and around 2 million deaths. The plants reviewed here provide potential leads for improving ORT formulations and properties.

2. Mode of action of enterotoxic bacteria

AB_5 enterotoxins are originally encoded by prophages STX1 or STX2 of *Shigella* sp. (Herold, 2005), or by phage CTXφ of *Vibrio* sp. (McCloud, 2004). Strains of the bacteria that do not express enterotoxins, which are a majority, are usually benign. Phages can undergo horizontal transfer and infect/transfer the toxin to other bacterial species and this is the case for the STX1 and STX2 prophages with *E. coli*. The primary role of AB_5 molecules is not known. Recently it's been suggested that AB_5 enterotoxin is a weapon against protozoa and immune system cells such as nucleophiles and their production is activated by reactive oxygen species.

Enterotoxic bacterial infection proceeds from the ingestion of contaminated water or food. Sufficient numbers of bacteria must survive passage through the stomach and into the intestine, where they anchor to the intestinal wall. Here, they colonize the intestinal wall and grow without any great ill-effects to the host. Once the bacterial colony reaches a certain number of cells, enterotoxin production is switched on. The assembled enterotoxin is secreted via the type II secretion system. Recent developments in research on strategies of *V. cholerae* to maintain fitness in different ecological niches and protein production are described in a recent review by Sikora (Sikora, 2013).

Cholera is a model bacterial disease caused by microorganisms producing AB_5-type enterotoxins (Beddoe *et al.*, 2010). The molecular structure of the toxin is formed from a proper toxin – A subunit and donut-like ring of five B-subunits, Figure 1. The B-subunits, which recognize GM_1 gangliosides in human cells, act as a chaperone/delivery vehicle for the A-subunit

A)

```
  1 mvkiifvffi flssfsyand dklyradsrp pdeikqsggl mprgqseyfd rgtqmninly
 61 dhargtqtgf vrhddgyvst sislrsahlv gqtilsghst yyiyviatap nmfnvndvlg
121 aysphpdeqe vsalggipys qiygwyrvhf gvldeqlhrn rgyrdryysn ldiapaadgy
181 glagfppehr awreepwihh appgcgnapr ssmsntcdek tqslgvkfld eyqskvkrqi
241 fsgyqsdidt hnrikdel
```

B)

```
  1 miklkfgvff tvllssayah gtpqnitdlc aeyhntqiyt lndkifsyte slagkremai
 61 itfkngaifq vevpgsqhid sqkkaiermk dtlriaylte akveklcvwn nktphaiaai
121 sman
```

C)

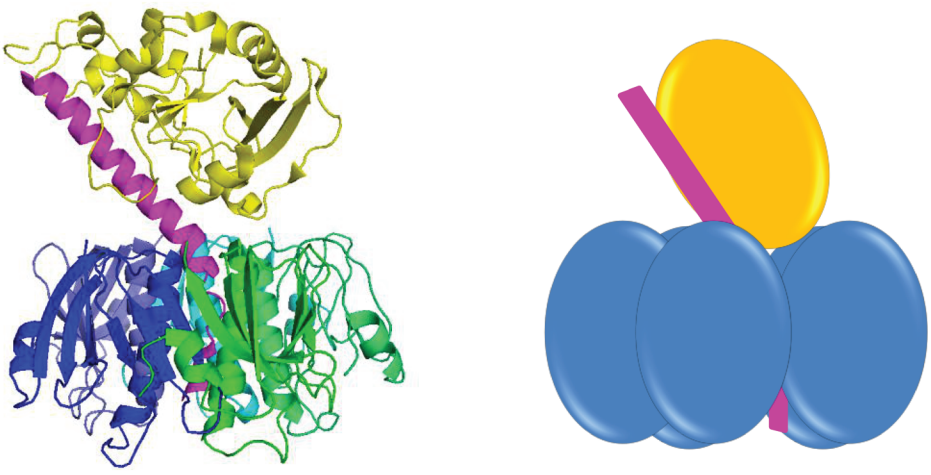

Fig. 1. Primary and 3D structural data for cholera toxin (PDB accession code 1XTC).

A) Protein sequence of cholera toxin subunit A. The first 18 residues (italics) constitute a signal sequence that is removed during secretion. The A1 domain is separated from the A2 domain by 'nicking' – an exogenous human protease breaks the backbone chain between residue Ser212 and Met213. The A2 domain is denoted in italics and bold text. The A1 and A2 remain joined through a Cys-Cys linkage denoted by a line. B) Protein sequence of the cholera toxin subunit B. The signal peptide (italics) is removed during secretion from *V. cholerae*. C) Structure of cholera toxin (PDB: 1XTC) as a cartoon representation of the secondary structure elements of cholera toxin. The five B-subunits are colored in green-blue shades. The A2 subunit (magenta) forms a long helical element that is embed in the center of the donut-shaped B-subunit pentamer. The A2 subunit is linked to the A1 subunit (yellow) through disulfide bonds. B) schematic representation of the enterotoxin structure. The GM_1-binding sites are situated in the B-subunits and on the opposite face of the A1 subunit.

that sits on the opposite face of the donut. The B-subunits of different enterotoxic bacteria have similar secondary and tertiary structures, as well as similar modes of binding to GM_1, although their primary amino acid sequences are quite distinct. The A-subunit is formed from two polypeptide chains: a long alpha-helical A2 domain that anchors the A1 domain into the hole of the B5 structure. The A1 domain, crosslinked to the A2 domain through disulfide bonds, is processed in the endoplasmic reticulum and released to the cytoplasm.

The introduction of the toxin to human cells follows a specific path involving initial endocytosis of the GM_1-toxin complex followed by transport to the Golgi and endoplasmic reticulum. The toxin is processed in the endoplasmic reticulum and the A1 subunit is released into the cytoplasm where subunit A1 binds to, and activates, ADP-ribosylation factor 6 (Arf6). This unwanted stimulation interferes with a range of cellular processes involving cAMP. In particular, the disregulation of cAMP levels results in the opening of ion channels and rapid loss of ions and water from the human cell which forms the diarrheal episode (Kopic and Geibel, 2010).

The multi-step process of the bacterial life cycle, involvement of a bacteriophage, toxin production, transport and action provides several potential targets where the disease process can be mediated/modified. Plant extracts can inhibit the initial anchoring of bacteria to the digestive system (Birdi *et al.*, 2010), down-regulate toxin production in bacteria (Birdi *et al.*, 2010; Brijesh *et al.*, 2009), inhibit the binding of toxin to GM_1 (Birdi *et al.*, 2010; J.-C. Chen *et al.*, 2006; 2007; 2009;), close toxin-activated ion channels (Fischer *et al.*, 2004) etc.

Despite this, the majority of the reported research focuses almost exclusively on the antimicrobial properties of plants (Table I). However, it needs to be noted that concentration of studied antimicrobial plant extracts is usually much higher and expressed as minimal inhibitory concentration (MIC) in mg/ml range than those observed for antibiotics – usually in µg/ml (http://www.eucast.org/mic_distributions/).

Plants have been used as a treatment and source of active substances for millennia with Ayurvedic practice and the roots of Chinese medicine providing the best established, documented and known examples. Ethnopharmacists collect the remaining local folk knowledge in various worldwide regions such as India (Dey and De, 2012; Tetali *et al.*, 2009), Indonesia (Grosvenor, Supriono and Gray, 1995) or Nigeria (Tekwu, Pieme and Beng, 2012). In the majority of cases, little or no scientific validation has been provided for the efficacy of plant extracts against disease. Experienced practitioners of plant medicine take careful note on the method of collection and storage of the plant material, as well as the preparation and use of the plant extract. Still, there is often a lack of validated identification of the bacterial species, treatment process, plant used, or use of placebo controls. This is best exemplified by making a simple internet search that often results in general list of diseases that can be cured by a particular plant, very often without any useful details on how to prepare or use the plant material.

Scientists have validated the beneficial effects of many plant extracts, including those against enterotoxic bacteria, particularly the more common and readily available plants. The antimicrobial active ingredients are often hydrophobic in nature and are most efficiently obtained by organic solvent extraction such as ethanol, methanol or acetone requiring a chemical laboratory (*e.g.* Rajan, Thirunalasundari and Jeeva, 2011). Data are also collected for simple aqueous extracts or decoctions – basically equivalent to a cup of herbal tea or broth. While ethanol extracts might support the effects of alcoholic beverages popularly used as digestives, they are clearly unsuitable for children less than five years old who represent one of the most sensitive groups of diarrhea patients. The use of methanol or acetone as a solvent raises safety issues. While the production of essential oils through steam distillation is an energy and time consuming process, unwanted solvents can be avoided and a safer product can be produced. They also potentially work in much lower concentrations then aqueous extracts and so they make an interesting alternative. Still, in our opinion, the ideal, active plant extracts should be available from a simple cold or hot water extract. Considering this point, the reviewed literature is focused towards the use of aqueous extracts.

3. Examples of active plants, plant parts and mode of preparation

Folk medicine provides the starting point for many investigations and the selection of plants and part of the plants to study. However, we must recognize that the properties of plants that have been cultivated and selected over the millennia may change. For example 1000 year old Anglo-Saxon recipes were used to test the antibacterial properties of plants such as *Potentilla reptans* (European cinquefoil) against wound infections and modern experiments found that plant extracts have stronger antimicrobial properties against gram negative intestinal bacteria such as *E. coli* than wound-infecting bacteria (*S. aureus*) (Watkins *et al.*, 2012). This makes the extracts useful for a possible treatment of diarrhea, and maybe less suitable in their original use to treat skin lesions.

Indian Ayurvedic medicine is based on phytomedicinal properties practiced over several millennia. Studies on Ayurvedic recipes revealed that an aqueous

Table I
Summary of antimicrobial properties of various plant extracts

Region	Plant		Method of preparation	Tested enteropathogens	Assay	Result	Reference
	plant name	common name (plant's part)					
Africa (Cameroon)	*Albizia gummifera*	(leaves)	Hexane, ethyl acetate and methanol (MeOH) extracts	*E. coli* LMP0101U, *S. dysenteriae* LMP0208U, *S. flexneri* LMP0313U	1. Agar disc diffusion 2. Broth microdilution method (MIC) with 0,5% phenol red	The MIC is dependent on the solvents and is between 0.032 (MeOH *N. latifoli* bark extr.) and 0.512 mg/ml.	Tekwu *et al.*, 2012
	Ficus exasperata *Nauclea latifoli*	(leaves and bark) (leaves and bark)					
South Africa	*Elephantorrhiza burkei*	(stem rhizome)	methanol, acetone, ethanol and boiling water extracts	*V. cholerae, E. coli* ATCC 35218, *Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Shihella boydii*	1. Agar-well diffusion 2. Broth microdilution (MIC)	The MIC is dependent on plant extract preparation and bacte rial strain. All of the plants have antimicrobial activities 0.039–0.312 mg/ml. against *V. cholerae, S. dysenteriae* and *S. flexneri*. *E.coli* is resistant to *E. burkei, E. elephantina, S. brachypetala* and *S. cordatum*	Mathabe *et al.*, 2006
	Elephantorrhiza elephantina	(stem rhizome)					
	Gymnosporia senegalensis	(roots)					
	Indigofera daleoides	(whole plant)					
	Ozoroa insignis	(stem bark)					
	Spirostachys africana	(stem bark)					
	Schotia brachypetala	Huilboerboon (stem bark)					
	Syzygium cordatum	Water berry (stem bark)					
	Ximenia caffra	(stem bark)					
	Eucalyptus dives	Eucalyptus	Essential oil (EO)	*E.coli* O157:H7, *Lactobacillus* sp.	Broth microdilution with 0.15% agar	EO from eucalyptus has very similar activity to coriander seeds (0.2% vol/vol).	Delaquis *et al.*, 2002
South America	*Eugenia uniflora*	Brazilian Cherry	Essential oil and methanol extract	Sixteen *E. coli* strains isolated from human specimens in the Clinical Hospital of Botucatu Medical School	Agar dilution method	MIC MeOH = 15.9 mg/ml MIC EO = 27.6 mg/ml	Silva *et al.*, 2012
	Baccharis dracunculifolia	–				MIC MeOH = 32.4 mg/ml MIC EO = 25.8 mg/ml	
	Vernonia polyanthes	–				MIC MeOH = 26.9 mg/ml MIC EO =24.1 mg/ml	
Asia (Bangladesh)	*Psidium guajava*	Guava (leaves and bark)	Methanol extract and crude aqueous mixture	*V. cholerae* O1	1. Agar diffusion method 2. Microdilution method (MIC and MBC)	MBC: crude preparation – 1.25mg/ml and methanol extract – 0.85 mg/ml	Rahim *et al.*, 2010

Table I continued

Region	Plant		Method of preparation	Tested enteropathogens	Assay	Result	Reference
	plant name	common name (plant's part)					
North America (Caribbean)	*Origanum vulgare*	oregano	Essential oil	*E.coli* O157:H7	1. Disc diffusion 2. Broth microdilution (MIC and MBC)	EO has antibacterial activity against *E. coli* (max. MBC – 0.08–2.5 µl/ml)	Burt and Reinders, 2003
	Thymus vulgaris	thyme					
North America (Mexico)	*Caesalpinia pulcherrima*	Poinciana	Methanol and aqueous extracts	*E. coli* ATCC 25922 (control), clinical isolates of *E. coli* 0157:H7, *S. ssonnei* and *S. flexneri*	Agar dilulution (1, 2, 4 and 8 mg/ml)	These plant extracts were tested at concentration of 8 mg/ml and found to inhibit the growth of tested strains. Methanol extracts have better antimicrobial activities than aqueous solutions	Alanís, *et al.*, 2005
	Chiranthodendron pentadactylon	Mexican hand tree					
	Chrysactinia mexicana	–					
	Geranium mexicanum	(roots and aerial part)					
	Punica granatum	pomegranate					
Asia (Sri Lanka)	*Cinnamomum zeylanicum*	cinnamon (bark)	water, ethanol and petroleum ether-based extracts, essential oil	*E. coli* O157:H7, *E. coli* ATCC 25921, *E. coli* ATCC25922 and *E. coli* ATCC11105	1. *E. coli* was cultured with three different concentrations of cinnamon extract. 2. Identification of the anti-microbial compound 3. Broth microdilution(MIC) 4. Disc diffusion	Cinnamon bark has antibacterial activity against *E. coli*. The antibacterial compound is cinnamic aldehyde.	Muthuswamy, *et al.*, 2007 Senhaji, *et al.*, 2007
Asia	*Tamarindus indica*	Tamarind (leaves, stemp bark, fruit pulp)	Ethanol, hot and cold water extracts	*E. coli* from diarrheal stools (7 strains) *E. coli* ATCC 11775	1. Agar diffusion method 2. Macrodilution method (MIC and MBC)	MBC fruit and bark = = 125 mg/ml. Only one *E. coli* strain was resistant to 250 mg/ml tamarind extract. Leaves have the lowest antimicrobial activity.	Nwodo, *et al.*, 2011
	Mangifera indica	Mango (kernel)	Ethanol (EtOH) and aqueous extract	*S. dysenteriae*	1. Disc diffusion method 2. Agar dilution method	MIC: aq extr. – 0.38 mg/ml, EtOH extr. – 0.19mg/ml	Rajan *et al.*, 2011
	Allium sativum	Garlic	Water extract	*E. coli* O157	1. Growth of *E. coli* on agar plates containing 1–2% of garlic extract 2. *E. coli* O/N culture was added to garlic extract and the number of living cells was counted on agar plates	Garlic has antimicrobial activity against *E. coli* O157. Fresh garlic killed *E. coli* much faster than 1 year old garlic powder.	Sasaki *et al.*, 1999

Table I continued

Region	Plant		Method of preparation	Tested enteropathogens	Assay	Result	Reference
	plant name	common name (plant's part)					
Asia	*Curcuma longa*	Tumeric leaves	Methanol extract	*E. coil* AM 8/98, *S. dysenteriae* 1, 2 and 6, *E. coli* VC sonawave 3:37C, *E. coli* CD/99/1, *V. cholerae* 865, *Lactobacillus arabinosus* CD /99/1	1. Agar dilution method 2. Disc diffusion	MIC: *E. coil* AM 8/98 – 2–5 µg/ml; *S. dysenteriae*, *E. coli* VC sonawave 3:37C and *E. coli* CD/99/1 – 150 µg/ml; *V. cholerae* – 100–150 µg/ml; *L.arabinosus* –150–200 µg/ml	Mazumder R., *et al.*
	Punica granatum	(rind and roots)	methanol, acetone, ethanol and boiling water extracts	*E. coli* UP 2566, *S. dysenteriae* IOA-108, *V. cholerae*, *E. coli* ATCC 35218	1. Agar well diffusion method (150 mg/ml) 2. Broth microdilution (MIC)	The lowest activity against tested bacteria with water extract (MIC: 0.156–0.3 mg/ml). With other solvents – MIC: 0.078 mg/ml	Mathabe *et al.*, 2006
	Camellia sinensis	tea (leaves)	Alcohol extract – dried and brown powder was used	111 baterial strains were tested including: 15 *E. coli*, 24 *Shigella* spp. and 23 *V. cholerae*	Agar dilution method	Tea has antimicrobial activity against studied bacteria. Growth of most *E. coli* strains was inhibited between 10–20 µg/ml; *Shigella* spp.: 20–30 µg/ml and *V.cholerae*: 10–30 µg/ml.	Bandyo-padhyay *et al.*, 2005
	Cuminum cyminum	Cumin (seeds)	Essential oil	*E. coli* ATCC 35218 and 11 *Vibrio* spp. strains	1. Disc diffusion 2. Brothmicro dilution (MIC and MBC)	MBC: *E. coli* – 0.625 mg/ml and *Vibrio* spp. – 0.31–1.25 mg/ml	Hajlaoui *et al.*, 2010
Asia (India)	*Azadirachta indica*	neem (leaves)	methanol extract	*V. cholerae* (NB2 and SG24) – belonged to O1 and O139, PC4, PC9, PC11, PC14 serotypes	1. Disc diffusion 2. Broth microdilution (MIC and MBC)	MBC = 10 mg/ml MIC > 5 mg/ml	Thakurta P. *et al.*
	Illicium verum	Star anise	ethanol and petroleum extracts	*E. coli*	Standard agar cup plate method – active coumpounds were studied	Antimicrobial activity of star anise is mainly due to anethole	De *et al.*, 2002

Table I continued

Region	Plant		Method of preparation	Tested enteropathogens	Assay	Result	Reference
	plant name	common name (plant's part)					
Asia (India	*Ocimum sanctum*	Holy Basil (whole plant)	70% alcohol extract	*E. coli* UP 2566, *S. dysenteriae* IOA-108	Agar well diffusion method (150 mg/ml) – 45 Indian plants were tested	150 mg/ml of plants extracts can inhibit growth of *S. dysenteriae* and *E. coli*. Extracts work better against *Shigella*.	Ahmad and Beg 2001
	Morus alba	White mulberry (leaves)					
	Hemidesmus indicus	Indian sarsa-parilla (roots)					
	Eucalyptus sp.	Eucalyptus leaves)					
	Casuarina equisetifolia	She-oak (bark and leaves)					
	Zizyphus jujuba	Jujube (leaves)				150 mg/ml of plants extracts with similar efficacy can inhibit growth of *E. coli* and *S. dysenteriae*	
	Syzgium cumini	Jamun (bark and leaves)					
	Syzygium aromaticum	Clove (bud and oil)					
	Saussurea lappa	(roots)					
	Acorus calamus	Sweet flag (rhizome)				150 mg/ml of plants extracts can inhibit growth of *S.dysenteriae* and *E. coli.* But ork better against *E. coli*.	
	Allium cepa	Onion (leaves)					
	Camelia sinensis	Tea (leaves)					
Europe	*Vaccinium myrtillus*	Blueberry (berries)	Lyophilized berry extract and phenolic compounds	*E. coli* ATTC 11775, *E. coli* CM871	1. Agar diffusion method 2. Bacteria growth curve *Lactobacillus* sp. (7 strains) measurement – bacteria grew with 0.5, 1 or 5 mg/ml berry extracts	Finnish berry extracts (1 mg/ml) have animicrobial activity against *E. coli* (except Blackcurrant to ATTC 11775). All studied probiotic bacteria are resistant to these extracts	Puupponen--Pimiä *et al.*, 2001
	Rubus idaeus	Raspberry (berries)					
	Vaccinium vitisidaca	Lingonberry (berries)					
	Ribes nigrum	Blackcurrant (berries)					
	Rubus chamaemorus	Cloudberry (berries)					
	Vaccinium oxycoccus	Cranberry (berries)					
	Hippophae rhamnoides	Sea buckthorn berry (berries)					
	Fragaria ananassa	Strawberry (berries)					

Table I continued

Region	Plant		Method of preparation	Tested enteropathogens	Assay	Result	Reference
	plant name	common name (plant's part)					
Europe	*Mentha pulegium*	Squaw Mint (flowering aerial parts)	Essential oil	*E. coli* ATCC 8739, *V. cholerae* Inaba	1. Disc diffusion 2. Broth microdilution (MIC and MBC)	MBC: *E. coli* – 4 µl/ml and and *V. cholerae* – 1 µl/m	Mahboubi and Haghi, 2008
	Agrimonia eupatoria	Agrimony (aerial parts and roots)	Red wine, ethanol (25% or 75%) and boiling water extracts (aq)	*E. coli* (UEL 57)	Microdilution method (MIC)	0.2 mg/ml of root aq and EtOH. extract inhibit >60% *E. coli* growth	Watkins *et al.*, 2012
	Potentilla reptans	Creeping cinquefoil (aerial parts and roots)					
	Thymus vulgaris	Thyme	Essential oil, methanol and aqueous extracts	*E. coli* O157:H7, *E. coli* ATTC 25922 (control), *S. sonnei*–1,2 and *S. flexneri*–1,2	1. Disc diffusion 2. Broth microdilution (MIC and MBC) 3. Agar dilulution (1, 2, 4 and 8 mg/ml)	Methanol extract has better antimicrobial activity than aqueous. Methanol extract of thyme inhibits 100% growth of all tested strains.	Burt and Reinders, 2003
Europe/Asia	*Anethum graveolens*	Dill	Essential oil	*E. coli* O157:H7, *Lactobacillus* sp.	Broth microdilution with 0.15% agar	EO from coriander leaves have almost two times less antimicrobial activity than seeds (0.4%vol/vol) and is similar to dill. (0.2%vol/vol).	Delaquis *et al.*, 2002
	Coriandrum sativum	Coriander (leaves and seeds)					
	Matricaria chamomilla	Chamomile	Essential oil and methanol extract	Sixteen *E. coli* strains isolated from human specimens in the Clinical Hospital of Botucatu Medical School	Agar dilution method	MIC EtOH = 43.4 mg/ml MIC EO = 28.2 mg/ml	Silva *et al.*, 2012
	Nepeta cataria	Catnip (plants at flowering stage)	Methanol extract and essential oil	*E. coli* A1, *Shigella* spp., *E. coli* ATCC 43894	1. Disc diffusion 2. Broth microdilution (MIC and MBC)	MIC MeOH: 31.25 µg/ml MIC EO: 125µg/ml	Adiguzel *et al.*, 2009 Zomorodian *et al.*, 2012
	Origanum vulgare	Oregano	Essential oil	*E. coli* O157:H7	1. Disc diffusion 2. Broth microdilution (MIC and MBC)	EO has antibacterial activity against *E. coli*	Burt and Reinders, 2003

decoction of guava leaves (*Psidium guajava*) and unripe bael fruit (*Aegle marmelos*) was found to have weak bacteriostatic/bacteriocidal properties (Birdi *et al.*, 2010; Brijesh *et al.*, 2009). The study also included tests of adherence/invasion potential of different bacterial species producing enterotoxins to human cells in the presence of plant extract. Because extracts of both plants inhibited the adherence and invasion of bacteria, they could play a positive role in the treatment of bacterial infection by inhibiting the initial colonization of the digestive system by the bacteria. This is a very important but rarely studied phenomena despite the fact that disruption of attachment – the first stage of bacterial infection – is a key property to prevent disease onset. Finding and using plants with this properties could be crucial in case of infections caused by hemolytic strains causing bloody diarrhea that are the most damaging and difficult forms of diarrhea to treat, requiring hospitalization, antibiotic treatment and which cannot currently be alleviated by ORT alone.

The method in which plant extracts are prepared contributes greatly to its efficacy. Prolonged heat treatment might degrade active ingredients present in the initial plant extract or lead to chemical modifications and an increase in the concentration of active ingredients. Examples of different treatment outcomes depending on the extract preparation were described by Rahim *et al.* and Birdi (Rahim *et al.*, 2010; Birdi *et al.*, 2010). Birdi *et al.* found that extract of guava (*P. guajava*) leaves were more effective against antibiotic-resistant *V. cholerae* strains than Birdi, however, Rahim prepared a cold water extract of the guava leaves whereas Birdi heated and reduced the plant extracts (Rahim *et al.*, 2010; Birdi *et al.*, 2010). This suggests that heating may destroy part of the antibacterial properties of the guava leaves although Rahim *et al.* did not find that heating of their samples reduced their efficacy (Rahim *et al.*, 2010). Variable antimicrobial properties of particular plant extracts can be commonly found in the literature, which stresses the fact that careful attention must be paid to sample collection, storage and preparation, as well as the bacteria strains used in subsequent studies.

The specificity of antimicrobial activities of plant extracts towards pathogenic bacterial strains as compared with benign strains are rarely tested. An example of a study addressing this issue is the test of 26 Mexican plant extracts against non-pathogenic *E. coli* and the enterotoxic *E. coli* O157:H7, amongst others (Alanís *et al.*, 2005). The aqueous extracts used in these studies showed a much better specificity for the pathogenic *E. coli* O157:H7 strain over a non-pathogenic *E. coli* strain when compared to methanolic extracts. *Carica papaya* (papaya), *Ocimum basilicum* (basil), *Matricaria chamomilla* (chamomile) and *Thymus vulgaris* (thyme) are commonly known plants reported with such antimicrobial specificity. Patient recovery rates and reestablishing gut flora, which can take several weeks following hospitalization, are an important consideration with respect to diarrhea. The retention of any beneficial bacteria in the digestive system by the selective action of plant extracts has much to be recommended.

Organic solvents, chiefly ethanol and methanol, are often used to obtain active ingredients from plant extracts but using them in the treatment of patients with acute diarrhea is highly questionable. A refluxed ethanolic extract of cinnamon (*Cinnamomum zeylanicum*) inhibited the growth of *Listeria inocua* and *E. coli* O157:H7 but an aqueous extract was ineffective (Muthuswamy, Rupasinghe and Stratton, 2007). The 50-fold dilution of the ethanolic extracts means that the final solution still contains 2% ethanol, which is unsuitable for the treatment of dehydrated patients, particularly children. Senhaji found that the essential oil of the same plant, obtained without organic solvents, was effective against *E. coli* O157:H7 (Senhaji, Faid and Kalalou, 2007). In this case pure essential oil could be a second choice for studies, after simple water extracts.

Plant metabolite concentrations often vary during the growth cycle and in different parts of the plant so a consistent level of therapeutic molecules is an important consideration. For *Nepeta cataria* (catnip), the antibacterial properties of essential oils distilled at three different stages of plant growth were tested (Zomorodian *et al.*, 2012). In this case, consistent results were obtained across the growth stages which uphold specificity for *S. aureus* and *Shigella* sp. over *E. coli*. It is worth noting that the essential oil content of catnip tea will be much lower than the levels of essential oils reported to have an antibacterial effect and also that catnip tea is not recommended for women during pregnancy or lactation (Ernst, 2002).

A range of European berries (blueberry, raspberry, lingonberry, blackcurrant, strawberry, cloud berry, sea buckthorn berry and cranberry) were found to be more active against *E. coli* and other pathogenic bacteria compared to *Lactobacillus* sp. (Puupponen-Pimiä *et al.*, 2001). However, the samples were first extracted with 70% acetone and processed to remove sugars. It is not possible to estimate the amount of fruit required providing equivalent levels of active material and if a low concentration of fruit extract could replace part of the sugar component in ORT. Positive data for raspberry fruit and cordial at 10% dilution has also been reported (Ryan, Wilkinson and Cavanagh, 2001). While neat fruit juices are generally not recommended for the treatment of diarrhea, these studies support the potential inclusion of natural, rather than artificial, fruit flavors in ORT formulas.

Publications naturally report positive antimicrobial properties of plant extracts but it is not always easy to

gauge their effectiveness. Plant extract yields and other essential data are often unreported and this adds to difficulties in comparing the different experimental methods used between publications. Mathabe surveyed the effect of plant extracts on several bacterial species, including *V. cholerae* and *Shigella* sp. (Mathabe *et al.*, 2006). They used extracts from different parts of 21, locally used plants. *Punnica granatum* (pomegranate), as clearly stated in the abstract, was the best known plant to give positive data. However, it is only on reading the full paper that it is pomegranate root that provided the positive data and not the juice or fruit. The reported MIC for the hot water extract of pomegranate root (0.156 μg/ml) is a lower concentration than would be expected from a teabag (1–2 g of plant material) in a mug of hot water (about a third of a liter) although exact extract yields were not reported. The cold water extract of tamarind (*Tamarindus indica*) fruit was very effective against a range of bacteria, including clinical isolates of *E. coli* that caused infantile diarrhea (Nwodo *et al.*, 2011). A cold water extract from 1 g plant material (1 teabag) per liter of water (about 3 mugs) was sufficient to achieve a bacteriocidal effect against *E. coli* (Nwodo *et al.*, 2011). Extracts of mango kernel (*Mangifera indica*), including a cold water extract, were effective against *S. dysenteriae* (Rajan, Thirunalasundari and Jeeva, 2011). The abstract of this paper is unambiguous as it states clearly that the data were obtained with mango kernel. The cold water extract was effective at a concentration of 1.5 g dry plant material per liter of water (Rajan, Thirunalasundari and Jeeva, 2011). Hajlaoui found that essential oil from cumin (*Cuminum cyminum*) was active against *Vibrio* sp. and *E. coli* (Hajlaoui *et al.*, 2010). However, the extraction yields and MICs suggest a high concentration of plant material is required to obtain an effective extraction (> 15 g/liter means at least three teabags in a mug of water).

Pairs of aqueous plant extracts can have a greater efficacy than their single counterparts (Han and Guo, 2012). The common traditional Chinese medicine pair of *Angelica sinensis* and *Sophora flavescens* was tested against four different bacteria. Neither plant had significant antibacterial properties when used alone but did so when used together. Specific interactions and effects of foods on pharmaceutical action are known. The adverse effect of grapefruit juice on a wide range of drugs is well documented (Hanley *et al.*, 2011).

Essential oils of thyme and oregano were effective against *E. coli* O157:H7 in disk diffusion and microplate (MIC) assays although this was the only bacterium tested (Burt and Reinders, 2003). This means that we cannot rule out a general antimicrobial effect that will also kill beneficial bacteria in the digestive tract. The principal ingredient of thyme oil, thymol, is used in mouthwashes as an antibacterial and antifungal agent but there are no safe recommendations about its use in food. Oregano oil is generally recognized as safe by the FDA at a consumption level of 200 mg a day (Food. Listing of Food Additive Status Part II. US Food and Drug Administration Web Site. http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/ucm191033.htm#ftnO). The bacteriostatic and bacteriocidal properties of oregano varied with the presence of other added ingredients (agar and soy lecithin).

Garlic is considered beneficial for a wide range of medical problems. Included as a 1% additive to media, garlic was active against several bacteria, including *E. coli* O157:H7 (Sasaki *et al.*, 1999). Fresh garlic extract and allicin, the principal compound in garlic suspected of carrying antimicrobial properties, had equal or better activity than five tested antibiotics against a range of antibiotic resistant strains of *Shigella* sp., eneterotoxic *E. coli* and *V. cholerae*. (Ahsan *et al.*, 1996). Allicin is an unstable molecule and the effectiveness of garlic is highly dependent on the source of the plant material, method of preparation, as well as the ages of the plant material and extract. Allicin is not the only active component of garlic, Politi *et al.* identified a polysaccharide component isolated from garlic that binds to the B-subunit of cholera toxin to confer antitoxin properties (Politi *et al.* 2006).

4. Avenues for future research

The majority of published works in the field focus on the antimicrobial properties of plant extracts. Few studies test the specificity of plant extracts for infectious over benign bacterial strains. The toxicity of plant extracts to humans should also be considered, particularly when highly concentrated fractions are used, or parts of the plant that are not normally ingested. Pharmaceutical practice focuses on developing one drug or treatment to target a specific and weak point in the disease mechanism. In principal, a mixture of plant extracts may present a synergistic effect against several stages of the infection cycle, specificities against a wide range of bacteria and overall lead to obtaining a more effective remedy for diarrhea.

The search for beneficial plant extracts would benefit from multi-disciplinary, collaborative, large-scale screening approaches. The first task is to select plant materials that are generally considered safe for human consumption (including children and pregnant/lactating women) as based on literature searches. Plant material should be collected at different growth stages and from different varieties of plant. Screening of fresh, stored and dried plant materials should be performed. Plant extracts should be prepared with cold water, hot

water and boiling/refluxing extractions – toxic solvents should be avoided. The long-term stability of the extracts in solution and/or as freeze-dried powders should be compared.

Antimicrobial screens should be based around well-established protocols to measure bacteriostatic and bacteriocidal properties of the plant extracts. However, it is important to test a range of enterotoxic and benign strains of bacteria in order to obtain ideas on the specificity of plant extracts. Advanced protocols based on mammalian cell cultures to test the anti-adhesion and anti-invasion properties of plant extracts on bacterial cell cultures are critical to the discovery of plant extracts that can counter hemolytic strains of enterotoxic bacteria. Protocols to measure the downregulation of toxin production (rtPCR) and toxin secretion by plant extracts are worth carrying out to ascertain if plant extracts are capable of slowing diarrheal episodes.

Returning to sample selection, combinations of optimal plant extracts should be tested in combination to ensure that their full activity is maintained or synergy obtained. The final aim is to have ready-to use ORT in solution and sachets of dried ORT to be dissolved in clean water. These products fulfill the aim of treating diarrhea but prevention and control of the disease might be aided with sachets of dried plant extract (without added salt and sugar) or teabags filled with mixed plant materials.

The strategy outlined above means a change from the current approach of validating plant extracts on an individual or regional basis towards a more multidisciplinary, collaborative effort. We have recently instigated a projected targeted towards AB_5-producing bacteria, establishing a range of protocols in three different labs. Our antimicrobial data corroborate existing published data for a number of hot water plant extracts and reveal new, include toxicity studies on human cell lines and a number of anti-toxin properties. However, we still do not employ a full range of protocols or have instigated animal or human trials to test our suggested mixes of plant extracts. Furthermore, the AB_5 family of enterotoxins represents just one group of enterotoxic pathogens that can be studied using the same set of protocols.

5. Conclusion

It is not possible to compare datasets for a particular plant due to different plant sources, extract preparations and microbial screens. However, the wide variability indicates that these points are very important being very important in determining if a particular plant extract will be effective or not. A plant extract, or more likely a mixture of plant extracts, that provide a wide spectrum of antimicrobial and anti-toxin properties would provide a powerful boost to ORT formulations. It is important in such a case that a suitable antimicrobial screen is used to assay the plants before distribution or sale. A second point is that several screens are required to test plant effects against different aspects of microbial colonization, toxin release and toxicity. It is this point that is largely overlooked in favor of traditional, plate-based antimicrobial screens. Future studies would benefit from large-scale collaborative screening with an aim of improving ORT.

Acknowledgement

M.P. thanks the Fundaçao para a Ciência e a Tecnologia (FCT) for the postdoctoral grant (SFRH/BPD/65762/2009). P.G. thanks FCT for a Ciencia Fellowship. We acknowledge FCT and POCI, with co-participation of the European Regional Development Fund (FEDER), for financial support under project PTDC/QUI-BIQ/115298/2009. I.S. and M.P. were supported by the Polish-Portugal Executive Program for years 2011–2012 sponsored by the Polish Ministry of Science and Higher Education and by FCT. This work was also supported by FCT through grant no. PEst-OE/EQB/LA0004/2011.

Literature

Adiguzel A., H. Ozer, M. Sokmen, M. Gulluce, A. Sokmen, H. Kilic and F. Sahin. 2009. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *Pol. J. Microbiol.* 58: 69–76.

Baris Ozlem Ahmad I and A.Z. Beg. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J. Ethnopharmacol*. 74: 113–123.

Ahsan M. and A.K.A. Chowdhury. 1996. Garlic Extract and Allicin: Broad Spectrum Antibacterial Agents Effective Against Multiple Drug Resistant Strains of *Shigella dysenteriae* type *Escherichia coli* and *Vibrio cholerae*. *Phytother. Res.* 10: 329–331.

Alanís A.D., F. Calzada, J.A.Cervantes, J. Torres and G.M. Ceballos. 2005. Antibacterial Properties of Some Plants Used in Mexican Traditional Medicine for the Treatment of Gastrointestinal Disorders. *J. Ethnopharmacol.* 100: 153–157.

Anonymous. 2002. *WHO Drug Information*. 16: 121–122. http://apps.who.int/medicinedocs/pdf/s4950e/s4950e.pdf

Bandyopadhyay D., T.P. Chatterjee, A. Dasgupta, J. Lourduraja and S.G. Dastidar. 2005. *In vitro* and *in vivo* antimicrobial action of tea: the commonest beverage of Asia. *Biol. Pharm. Bull.* 28: 2125–2127.

Beddoe T., A.W. Paton, J. Le Nours, J. Rossjohn and J.C. Paton. 2010. Structure, Biological Functions and Applications of the AB_5 Toxins. *Trends Biochem. Sci.* 35: 411–418.

Birdi T., P. Daswani, S. Brijesh, P. Tetali, A. Natu and N. Antia. 2010. Newer Insights into the Mechanism of Action of *Psidium guajava L.* Leaves in Infectious Diarrhoea. *BMC Complement Altern. Med.* 10: 33.

Brijesh S., P. Daswani, P. Tetali, N. Antia and T. Birdi. 2009. Studies on the Antidiarrhoeal Activity of *Aegle marmelos* Unripe Fruit: Validating Its Traditional Usage. *BMC Complement. Altern. Med.* 9: 47.

Burt S.A. and R.D. Reinders. 2003. Antibacterial Activity of Selected Plant Essential Oils Against *Escherichia coli* O157:H7. *Lett. Appl. Microbiol.* 36:162–167.

Chen J.-C., T.-Y. Ho, Y.-S. Chang, S.-L. Wu and C.-Y. Hsiang. 2006. Anti-diarrheal Effect of *Galla chinensis* on the *Escherichia coli*

Heat-labile Enterotoxin and Ganglioside Interaction. *J. Ethnopharmacol.* 103: 385–391.

Chen J.-C., T.-Y. Ho, Y.-S. Chang, S.-L. Wu, C.-C. Li and C.-Y. Hsiang. 2009. Identification of *Escherichia coli* Enterotoxin Inhibitors from Traditional Medicinal Herbs by *in silico*, *in vitro*, and *in vivo* Analyses. *J. Ethnopharmacol.* 121: 372–378.

Chen J.-C., L.-J. Huang, S.-L. Wu, S.-C. Kuo, T.-Y. Ho and C.-Y. Hsiang. 2007. Ginger and Its Bioactive Component Inhibit Enterotoxigenic *Escherichia coli* Heat-labile Enterotoxin-induced Diarrhea in Mice. *J. Agric. Food Chem.* 55: 8390–8397.

De M., A.K. De, P. Sen and A.B. Banerjee. Antimicrobial Properties of Star Anise (*Illicium verum Hook f*). *Phytother. Res.* 16: 94–95.

Delaquis P.J., K. Stanich, B. Girard and G. Mazza. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol.* 74: 101–109.

Dey A. and J.N. De. 2012. Ethnobotanical Survey of Purulia District, West Bengal, India for Medicinal Plants Used Against Gastrointestinal Disorders. *J. Ethnopharmacol.* 143: 68–80.

Ernst E. 2002. Herbal Medicinal Products During Pregnancy: are they safe? *BJOG* 109: 227–235.

Fischer H., T.E. Machen, J.H. Widdicombe, T.J.S. Carlson, S.R. King, J.W.S. Chow and B. Illek. 2004. A Novel Extract SB-300 from the Stem Bark Latex of *Croton lechleri* Inhibits CFTR-mediated Chloride Secretion in Human Colonic Epithelial Cells. *J. Ethnopharmacol.* 93: 351–357.

Grosvenor P.W., A. Supriono and D. Gray. 1995. Medicinal Plants from Riau Province , Sumatra, Indonesia . Part 2: Antibacterial and Antifungal Activity. *J. Ethnopharmacol.* 45: 97–111.

Hajlaoui H., H. Mighri, E. Noumi, M. Snoussi, N. Trabelsi, R. Ksouri and A. Bakhrouf. 2010. Chemical Composition and Biological Activities of Tunisian *Cuminum cyminum L*. Essential Oil: a High Effectiveness Against *Vibrio* spp. Strains. *Food Chem. Toxicol.* 48: 2186–2192.

Han C. and J. Guo. 2012. Antibacterial and anti-inflammatory Activity of Traditional Chinese Herb Pairs, *Angelica sinensis* and *Sophora flavescens*. *Inflammation* 35: 913–919.

Hanley M.J., P. Cancalon, W.W. Widmer and D.J. Greenblatt. 2011. The Effect of Grapefruit juice on Drug Disposition. *Expert Opin. Drug. Metab. Toxicol.* 7:267–286.

Herold S., H. Karch and H. Schmidt. 2004. Shiga toxin-encoding bacteriophages-genomes in motion. *Int. J. Med. Microbiol.* 294: 115–121.

S. Kopic and J.P. Geibel. 2010. Toxin mediated diarrhea in the 21 century: the pathophysiology of intestinal ion transport in the course of ETEC, *V. cholerae* and rotavirus infection. *Toxins*. 2: 2132–2157.

Lainhart W., G. Stolfa and G. Koudelka. 2009. Shiga toxin as a bacterial defense against a eukaryotic predator, *Tetrahymena thermophila. J. Bacteriol.* 191: 5116–5122.

Mahboubi M. and G. Haghi. 2008. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *J. Ethnopharmacol.* 119:325–327.

Mathabe M.C., R.V. Nikolova, N. Lall and N.Z. Nyazema. 2006. Antibacterial Activities of Medicinal Plants Used for the Treatment of Diarrhoea in Limpopo Province, South Africa. *J. Ethnopharmacol.* 105: 286–293.

Mazumder R., T. Mendiratta, S.C. Mondal and A. Mazumder. 2000. Antimicrobial Potency of the Leaf – Stalk Extract of *Curcuma longa* (LINN). *Anc. Sci. Life.* 20: 92–96.

McLeod S.M., H.H. Kimsey, B.M. Davis and M.K. Waldor. 2005. CTXphi and *Vibrio cholerae*: exploring a newly recognized type of phage-host cell relationship. *Mol. Microbiol.* 57: 347–356.

Muthuswamy S., H.P.V. Rupasinghe and G.W. Stratton. 2007. Antimicrobial effect of Cinnamon Bark Extract on Fresh-Cut Apples. *J. Food Saf.* 28: 534–549.

Nwodo U.U., G.E. Obiiyeke, V.N. Chigor and A.I. Okoh. 2011. Assessment of *Tamarindus indica* Extracts for Antibacterial Activity. *Int. J. Mol. Sci.* 12: 6385–6396.

Politi M., J. Alvaro-Blanco, P. Groves, A. Prieto, J.A. Leal, F.J. Cañada, J. Jiménez-Barbero. 2006. Screening of Garlic Water Extract for Binding Activity with Cholera Toxin B Pentamer by NMR Spectroscopy – An Old Remedy Giving a New Surprise. *Eur. J. Org. Chem.* 9: 2067–2073.

Prins J. M., S.J.H. van Deventer, E.J. Kuijper, P. Speelman. 1994. Clinical Relevance of Antibiotic-Induced Endotoxin Release. *Antimicrob. Agents Chemother.* 38: 1211–1218.

Puupponen-Pimiä R., L. Nohynek, C. Meier, M. Kähkönen, M. Heinonen, A. Hopia and K.M. Oksman-Caldentey. 2001. Antimicrobial Properties of Phenolic Compounds from Berries. *J. Appl. Microbiol.* 90: 494–507.

Rahim N., D.J. Gomes, H. Watanabe, S.R. Rahman, C. Chomvarin, H.P. Endtz and M. Alam. 2010. Antibacterial Activity of *Psidium Guajava* Leaf and Bark Against Multidrug-resistant *Vibrio cholerae*: Implication for Cholera Control. *Jpn. J. Infect. Dis.* 63:271–274.

Rajan S., T. Thirunalasundari and S. Jeeva. 2011. Anti-enteric Bacterial Activity and Phytochemical Analysis of the Seed Kernel Extract of *Mangifera indica Linnaeus* Against *Shigella dysenteriae* (Shiga, Corrig.) Castellani and Chalmers. *Asian Pac. J. Trop. Med.* 4: 294–300.

Ryan T., J.M. Wilkinson and H.M. Cavanagh. 2001. Antibacterial Activity of Raspberry Cordial *in vitro. Res. Vet. Sci.* 71: 155–159.

Sasaki J.-I., K. Takeshi, I. Kunio, U. Hidemitsu and M. Hajime. 1999. Antibacterial Activity of Garlic Powder Against *Escherichia coli* O-157: H7. *J. Nutr. Sci. Vitaminol.* 45: 785–790.

Senhaji O., M. Faid and I. Kalalou. 2007. Inactivation of *Escherichia coli* O157:H7 by Essential Oil from *Cinnamomum Zeylanicum*. *Braz. J. Infect. Dis.* 11: 234–236.

Sikora A.E. 2013. Proteins Secreted via the Type II Secretion System: Smart Strategies of *Vibrio Cholerae* to Maintain Fitness in Different Ecological Niches. *PLoS Pathog.* 9: e1003126.

Silva N.C.C., L. Barbosa, L.N. Seito and A. Fernandes Jun. 2012. Antimicrobial activity and phytochemical analysis of crude extracts and essential oils from medicinal plants. *Nat. Prod. Res.* 26: 1510–1514.

Stolfa G. and G. Koudelka. 2013. Entry and Killing of *Tetrahymena thermophila* by Bacterially Produced Shiga Toxin. *mBio*. 4: 1–10

Tekwu E.M., A.C. Pieme and V.P. Beng. 2012. Investigations of Antimicrobial Activity of Some Cameroonian Medicinal Plant Extracts Against Bacteria and Yeast with Gastrointestinal Relevance. *J. Ethnopharmacol.* 142: 265–723.

Tetali P., C. Waghchaure, P.G. Daswani, N.H. Antia and T.J. Birdi. 2009. Ethnobotanical Survey of Antidiarrhoeal Plants of Parinche Valley, Pune District, Maharashtra, India. *J. Ethnopharmacol.* 123: 29–236.

Thakurta P., P. Bhowmik, S. Mukherjee, T.K. Hajra, A. Patra and P.K. Bag. 2007. Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India. *J. Ethnopharmacol.* 111: 607–612.

Watkins F., B. Pendry, A. Sanchez-Medina and O. Corcoran. 2012. Antimicrobial Assays of Three Native British Plants Used in Anglo-Saxon Medicine for Wound Healing Formulations in 10th Century England. *J. Ethnopharmacol.* 144: 408–415.

Zomorodian K., M.J. Saharkhiz, S. Shariati, K. Pakshir, M.J. Rahimi and R. Khashei. 2012. Chemical Composition and Antimicrobial Activities of Essential Oils from *Nepeta cataria L.* Against Common Causes of Food-Borne Infections. *ISRN Pharm.* 2012: 591953.