

## Mint Water - the Science Behind the Tradition !

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**Abstract:** When cultures of three common pathogens, namely *Salmonella infantis*, *Salmonella hadar* and *Pseudomonas aeruginosa* were added individually to a commercial sample of Mint Water at levels of ~ 500 colony-forming units ml<sup>-1</sup>, no viable cells could be detected after 10 minutes exposure. It is suggested that D-carvone is the active ingredient and that there is a sound scientific basis for the use of this traditional Middle Eastern remedy for bacterial infections.

**Key words:** Mint water, human pathogens, D-carvone

### Introduction

It is a widely held belief in the United Arab Emirates and in other countries in the Gulf Region that mint water is an excellent cure for sore throats or intestinal infections resulting from the consumption of contaminated food or water (Anonymous, 2003). Traditionally, inhabitants have made infusions of fresh mint in their own homes but, more recently, commercial products have appeared on the market. Whether or not the commercial products have the same antimicrobial properties as traditional remedy was considered to be a point of some interest and hence it was decided to check the efficacy of one commercial brand against some common pathogens.

### Materials and Methods

Portions of several identical colonies of *Salmonella infantis*, *Salmonella hadar* and *Pseudomonas aeruginosa* were removed from plates of Plate Count Agar (PCA - Oxoid Code No. CM463; Unipath, Basing stoke, Hants, UK) with a sterile loop to make a suspension in an ampule of sterile saline (2 ml, 8.5 g l<sup>-1</sup> NaCl). The optical density of the suspension was measured with the Densimat (bioMérieux, Basing stoke, Hants, UK) and the individual suspensions were prepared diluted to give approximately 1.5 x 10<sup>5</sup> colony-forming units (cfu) ml<sup>-1</sup>.

A series of flasks (in duplicate) were then set-up containing either sterile tap water (250 ml) or commercial Mint Water (250 ml) and, into each flask, was deposited an aliquot (1.0 ml) of one of the pure cultures to give a concentration of around 500 cfu ml<sup>-1</sup> of solution. At intervals of 5 min (Mint Water) and 2 h (tap water), the content of each flask was swirled gently and 2 x 0.5 ml amounts of Mint Water or 2 x 0.1 ml of tap water were spread onto two plates of PCA. The plates were incubated at 37 °C for 48 hours prior to counting. The procedure was repeated for each pathogen and the entire trial repeated twice.

**Analysis of Volatile Constituents:** Duplicate sub-samples (10 ml) were transferred to conical flasks (250

ml) and a Dreschel head was fitted. The flask was then placed in a water-bath at 40 °C for 1 hour and, at the same time, volatile compounds were swept with nitrogen (40 ml min<sup>-1</sup>) into a trap containing 85 Tenax TA (Scientific Glass Engineering Ltd., Ringwood, Australia); the internal standard was 1, 2-dichlorobenzene.

GC/MS analysis was carried out with an HP 5890 Series II gas chromatograph (Hewlett Packard Ltd., Bracknell, UK) and a BPX5 fused silica capillary column (50 m x 0.32 mm i.d; Varian-Chrompack UK Ltd., Surrey, UK) directly connected to the ion source of the spectrometer. The volatile compounds were thermally desorbed from the Tenax trap over a period of 10 min at 0 °C, after which, the oven temperature was programmed to rise from 60 to 280 °C at 4 °C min<sup>-1</sup>; the helium carrier gas velocity was fixed at 1 ml min<sup>-1</sup> (Nickerson and Likens, 1966). The temperature of the spectrometer was maintained constant at 280 °C and the temperature of the injector at 250 °C. The mass spectra were determined at 70 eV and analyses by the HP-Data Analysis System. In order to identify unknown components, the NIST Library of mass spectra and sub-sets was used. The linear retention indices (LRI) were also calculated for each peak using a series of hydrocarbons (C<sub>6</sub>-C<sub>25</sub>) as a reference for comparison with LRI values from the literature.

### Results and Discussion

An analysis of the product showed that D-carvone was one of the dominant components being derived from the mint and this pattern is in-line with the data published by Papademas and Robinson (2002) for *Mentha viridis* (Table 1). The average concentration of D-carvone was 600 mg l<sup>-1</sup>, while dihydrocarvone (~ 80 mg l<sup>-1</sup>) and eucalyptol (~ 150 mg l<sup>-1</sup>) were well represented. The eucalyptol and D-carvone could be important with respect to the attractive flavour of the Mint Water (Pigott *et al.*, 1998), as may compounds like  $\alpha$ -pinene and  $\beta$ -caryophyllene (Mariaca *et al.*, 1997; Papademas and Robinson, 2002).

However, while these latter compounds help to give the

Table 1: Some volatile flavour compounds (mg kg<sup>-1</sup> ± Standard Deviation) identified in dry mint (*Mentha viridis*); all figures as means of three determinations from a composite sample

Native compounds	
"-pinene	46.3 ± 4.6
\$-pinene	79.1 ± 6.6
Limonene	258.7 ± 2.9
Eucalyptol	614.4 ± 9.4
D-carvone	4,341.8 ± 24.5
Dihydrocarvone	406.8 ± 5.4
\$-caryophyllene	441.2 ± 8.6
\$-cubebene	364.3 ± 4.0

After: Papademas and Robinson (2002)

Table 2: Mean total colony counts (cfu ml<sup>-1</sup>) of some common pathogens suspended in Mint Water for the times (minutes) indicated

Time	<i>S. infantis</i>	<i>S. hadar</i>	<i>Ps. aeruginosa</i>
0	500	600	250
5	51	18	ND
10	< 10	ND	ND
15	ND	ND	ND
20	ND	ND	ND
30	ND	ND	ND

ND = Not Detected

Table 3: Mean total colony counts (cfu ml<sup>-1</sup>) of some common pathogens suspended in tap water for the times (hours) indicated

Time	<i>S. infantis</i>	<i>S. hadar</i>	<i>Ps. aeruginosa</i>
0	500	600	250
2	293	370	343
4	145	310	440
6	55	188	628
8	43	183	893
10	38	180	2,690

Mint Water a pleasing flavour, what is important in the present context is that components like D-carvone and limonene have bacteriocidal properties (Imai *et al.*, 2001). The results shown in Table 2 suggest that this view is valid for, by monitoring the populations of the bacterium at regular intervals, it was shown that all the cells of *S. infantis* were rendered non-viable in the Mint Water in about ten minutes. In tap water, by contrast, around 10% of the cells remained viable for up to ten hours (Table 3). *S. hadar* was even more susceptible to the components in Mint Water - not detectable after five minutes, while *Ps. aeruginosa* was inactivated within five minutes. In tap water, the population of *S. hadar* declined initially and then stabilized, while the cell count of *Ps. aeruginosa* showed a ten-fold increase at ten hours.

This devastating impact of the commercial Mint Water is rather more dramatic than might be expected from the results of Imai *et al.* (2001), but it is worth recording that the latter authors used different strains of bacteria, at

much higher cell counts and under different experimental conditions. However, what was exciting about the results of Imai *et al.* (2001) was the finding that a clinical strain of *Helicobacter pylori*, the organism alleged to be associated with the development of duodenal ulcers in humans, was very susceptible to both D-carvone and limonene at levels of 100 and 50 mg l<sup>-1</sup>, respectively. As around 100 mg of carvone could be anticipated to be present in a small glass (150 ml) of the commercial Mint Water used in this present study, regular consumers in the Middle East could well be spared the misery of duodenal ulcers - especially if the Mint water is drunk on an empty stomach so that the active ingredients are not adsorbed onto particles of food.

As people suffering from a food-borne infection will also tend to drink rather than eat, this traditional medication translated into the commercial form could prove really effective. Obviously it will be important that the manufacturers of the commercial product(s) monitor its quality with respect to the compounds derived from the mint but, given this proviso, it would be most interesting to test the action of Mint Water under clinical conditions.

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