Investigation of the Antimicrobial Activity of Essential Oils of Culinary and Medicinal Herbs and Spices against Selected Gastrointestinal Pathogens

Mike Chorlton, Eugene Rees, Chris Phillips, Tim Claypole, Nidhika Berry and Paula Row
Gastrointestinal micro-organisms cause many deaths per year

*Salmonella* species cause food poisoning due to poorly cooked chicken and eggs in the UK

There are thought to be 93.8 million cases of gastroenteritis from non-typhoidal *Salmonella* worldwide per year, and 155,000 deaths

Irritable Bowel Syndrome...

Affects 10 - 22% of the UK population, more women than men

Can persist for decades

Symptoms include:
• abdominal pain
• diarrhoea
• constipation
• bloating and wind

Responsible for approximately half of all out-patient visits to a gastroenterology department

IBS has recently been linked to Small Intestinal Bacterial Overgrowth, SIBO.

Normally the small intestine contains very few bacteria:

- Terminal jejenum: $10^{0-4}$ cfu/ml
- Proximal ileum: $10^{0-5}$ cfu/ml
- Terminal ileum: $10^{0-5}$ cfu/ml

In SIBO, bacterial numbers can rise to $10^{11}$ cfu/ml

SIBO is currently defined as $\geq 10^5$ colonic bacteria in the small intestine*

Normally, easily digestible starch is completely digested and absorbed in the proximal small intestine. Poorly digestible starch is fermented in the colon, to give gas.

In SIBO, easily digestible starch meets bacteria in the small intestine and is fermented to produce gas.

SIBO is diagnosed with a breath test

Patients drink a glass of water containing an indigestible sugar, e.g., lactulose. They breathe into a bag, and the sample is analysed for the presence of hydrogen and methane.

Hydrogen is associated with diarrhoea-prone IBS.

Methane is associated with constipation-prone IBS.
IBS has been linked to intestinal dysbiosis

A change in the bacterial species present, not the numbers

Specifically, culture of the faecal microbiota from patients with IBS revealed:

- Decreased lactobacilli and bifidobacteria
- Increased facultative anaerobes, mainly streptococci and E. coli
- Higher counts of anaerobes such as Clostridium

Antibiotics can improve IBS symptoms

In America, clinical trials have been conducted which showed that IBS can be treated with antibiotics such as:

- **Metronidazole** (a non-absorbed antibiotic)
- **Neomycin**
- **Rifaximin (Xifaxan 200)**

However, IBS symptoms can return

Rifaximin is in the rifamycin family, used to treat tuberculosis and *C. difficile*, - there is concern about antibiotic resistance

New treatments are urgently needed

Hypothesis:

That essential oils of culinary herbs, and herbal medicines that have long been used to treat digestive disorders, have antibacterial activity, which would make them useful for treating infection with gastrointestinal pathogens and IBS.
Peppermint is currently used to treat IBS

Colpermin and Mintec are enteric-coated peppermint oil tablets.

They uncoat in the later part of the ileum - so would probably bypass SIBO

The useful activity of peppermint in these tablets is most likely peppermint’s **antispasmodic** effect
Disc Diffusion Assay

1) Plate out a lawn of bacteria, or yeast

2) Add 10 µl essential oil to a small disc and place it on the lawn of bacteria

3) Grow the bacteria

(Disc diffusion: Dilruba Meah)
Aniseed, asafoetida, cinnamon, clove, garlic, lemon balm, lemon grass, May Chang, oregano, peppermint, rosemary, thyme and winter savory were all strongly antibacterial against the *E. coli* type strain.
Cinnamon and winter savory were strongly antibacterial. Aniseed, asafoetida, caraway, clove, coriander, lemon balm, May Chang, oregano, rosemary, tea tree and thyme were also effective.
Effect of oils on *Salmonella enterica*

Cinnamon and winter savory were strongly antibacterial.

Aniseed, asafoetida clove, lavender, lemon balm, May Chang, oregano, tea tree, and thyme were also effective.
Many essential oils inhibited the growth of *Clostridium difficile*

Cinnamon, clove, garlic, lemon balm, lemon grass, May Chang, oregano, peppermint, thyme and winter savory were the most potent
Effect of Essential oils on *Candida albicans*

Many essential oils were antimicrobial towards *C. albicans*. The most effective were asafoetida, garlic, lemon balm, lemongrass, May Chang, thyme and winter savory.
Antibacterial activity against *S. enterica* under aerobic and anaerobic conditions

The upper GI tract is an aerobic environment; the colon is anaerobic.

The best essential oils were effective against *S. enterica* under aerobic and anaerobic conditions, although there were slight differences.
Batch Testing

Wine buyers know that different wines have different characters.

The vintage and the *terroir* affect the taste, the *compounds present*.

The potential for batch to batch variation of herbal medicines is often ignored.

We have seen evidence of batch variation in antibacterial activity of the essential oils.

We have analysed some of the oils by Gas chromatography mass spectrometry.
Cinnamon A, the more active one, has cinnamaldehyde as expected. Cinnamon B (probably leaf oil) has Eugenol and is more like clove oil.
Oregano, Thyme and Winter savory oil share common components. They all contain thymol and cymene. Winter savory has carvacrol too.
Variation in Aniseed Essential oils

Aniseed A and Aniseed B are totally different

Aniseed A has the expected composition; B is the more active one.
Variation in Lemon Balm Essential oils

Lemon balm A had higher activity against *E. coli*

Lemon balm essential oil can be very variable, depending upon harvest time. Citral becomes converted to citronellal.
Summary of Results

Cinnamon and Winter savory were strongly antibacterial against all of the organisms.

Aniseed, asafoetida, clove, oregano and thyme were also effective against all the organisms.

Batch variation was seen in antimicrobial activity and chemical composition of the essential oils.

We have identified the main compounds present in these oils; we aim to test these in the assay singly.
What about toxicity? Herbs have long been used to treat digestive disorders

In her 1932 book, A Modern Herbal, Grieve refers to *Aqua mirabilis*:

“used on the continent as an aromatic water for the treatment of internal pains”

It consisted of 1 part each of cinnamon, fennel, lavender, peppermint, rosemary and sage essential oils, 350 parts spirit (alcohol) and 644 parts distilled water
In the 1700s, William Lewis wrote the New London Dispensatory...

The New Dispensatory containing I) the elements of pharmacy, and II) the materia medica

It also refers to *Aqua mirabilis*:

This one contained:

“celandine leaves, melilot flowers, cardamum seeds, cubebs, galingale, nutmegs, cloves, mace, ginger…”