Abstract
INTRODUCTION: Dysbiosis is associated with a number of gastrointestinal and systemic disorders. There is a need for selectively acting antimicrobial agents capable of inhibiting the growth of potentially pathogenic microorganisms, or those found to be out of balance, while not negatively impacting the bulk gastrointestinal tract microflora. OBJECTIVE: The purpose of this in vitro study is to examine the potential of a selection of essential oils as agents to treat dysbiosis. MATERIALS AND METHODS: Eight essential oils were examined using the agar dilution method, including Carum carvi, Citrus aurantium var. amara, Foeniculum vulgare dulce, Illicium verum, Lavandula angustifolia, Mentha arvensis, Mentha x piperita, and Trachyspermum copticum. Doubling dilutions of the essential oils were tested against 12 species of intestinal bacteria, which represent the major genera found in the human gastrointestinal tract (GIT). RESULTS: Carum carvi, Lavandula angustifolia, Trachyspermum copticum, and Citrus aurantium var. amara essential oils displayed the greatest degree of selectivity, inhibiting the growth of potential pathogens at concentrations that had no effect on the beneficial bacteria examined. CONCLUSION: The most promising essential oils for the treatment of intestinal dysbiosis are Carum carvi, Lavandula angustifolia, Trachyspermum copticum, and Citrus aurantium var. amara. The herbs from which these oils are derived have long been used in the treatment of gastrointestinal symptoms and the in vitro results of this study suggest that their ingestion will have little detrimental impact on beneficial members of the GIT microflora. More research is needed, however, to investigate tolerability and safety concerns, and verify the selective action of these agents.

Introduction
Intestinal dysbiosis has been defined as qualitative and quantitative changes in the gastrointestinal flora, their metabolic activities, and/or their local distribution that produces harmful effects on the host. Dysbiosis has been associated with a number of conditions, including atopic eczema, rheumatoid arthritis, inflammatory bowel disease, and irritable bowel syndrome (IBS).

Evidence suggests a possible etiological role for dysbiosis in IBS, including epidemiological studies that have found a significantly increased risk of IBS following antibiotic use and bacterial gastroenteritis. Other evidence comes from colonic fermentation studies, which have found patients with IBS produce significantly greater amounts of colonic hydrogen than healthy controls and have altered fecal short-chain fatty acid profiles.
There is also direct evidence that the gastrointestinal tract (GIT) microflora of IBS patients differs from that of healthy individuals. An older study found IBS patients have significantly fewer coliform bacteria, lactobacilli, and bifidobacteria than controls.7 These findings are supported by more recent studies that found lower fecal concentrations of bifidobacteria in IBS patients, as well as lower levels of lactobacilli in diarrhea-predominant IBS patients.8

Whether this dysbiosis plays a role in the symptomatology of IBS has not been conclusively proven. However, the efficacy of probiotic agents in treating this condition16-19 in combination with evidence outlined above suggests a possible etiological role.

Thus, there is a need for selectively acting antimicrobial agents capable of inhibiting the growth of potentially pathogenic microorganisms, or those found to be out of balance, while not negatively impacting the bulk GIT microflora. In addition, since such agents may be prescribed concurrent with probiotics, it is beneficial that the antimicrobial agent not interfere with the growth of the supplemented probiotic organisms (e.g., lactobacilli and bifidobacteria).

Objective

The objective of this study is to examine the potential of a selection of essential oils as agents to treat intestinal dysbiosis. The essential oils investigated were chosen from carminative herbs traditionally used in the treatment of gastrointestinal disorders, including Carum carvi (caraway), Citrus aurantium var. amara (bitter orange), Foeniculum vulgare dulce (sweet fennel), Illicium verum (star anise), Lavandula angustifolia (lavender), Mentha arvensis (Japanese peppermint), Mentha x piperita (peppermint), and Trachyspermum copticum (ajowan).

Materials and Methods

Essential Oils

Pure essential oils were purchased from two sources: New Directions (Sydney, NSW, Australia) and Sydney Essential Oil Company (Sydney, NSW, Australia). The essential oils purchased from New Directions included Carum carvi, Foeniculum vulgare dulce, Illicium verum, Mentha x piperita, and Trachyspermum copticum. Mentha arvensis, Lavandula angustifolia, and Citrus aurantium var. amara were sourced from Sydney Essential Oil Company.

Organisms and Growth Conditions

Microorganisms were obtained from the Australian Collection of Microorganisms, University of Queensland, with the exception of Bifidobacterium bifidum and Bifidobacterium longum, which were obtained from the CSIRO Starter Culture Collection. Organisms were as follows: Bacteroides fragilis ACM 4768, Candida albicans ACM 4574, Clostridium difficile ACM 5047, Clostridium perfringens ACM 5116, Enterococcus faecalis ACM 4769, Escherichia coli ACM 1083, Eubacterium limosum ACM 383, Lactobacillus acidophilus ACM 547, Lactobacillus plantarum ACM 1903, Bifidobacterium bifidum CSCC 1903, Bifidobacterium longum CSCC 5188, and Peptostreptococcus anaerobius ACM 5059. These organisms represent the major genera of microorganisms found in the human GIT.20

Organisms were maintained on Reinforced Clostridial Agar (Oxoid), Wilkens-Chalgren Anaerobe Agar (Oxoid), Mueller Hinton Agar (Oxoid), or DeMan Rogosa Sharpe Agar (Oxoid). Inoculum was prepared by suspending colonies from 24-72 hour cultures in sterile saline. Using a CrystalSpec Nephelometer™ (Becton Dickinson & Company, Maryland, USA) suspensions were standardized to a 0.5 McFarland standard, giving ~10⁶ colony forming units (CFU) per mL for the bacteria and 10⁵ CFU per mL for Candida albicans. Aerobic bacteria were diluted 1:10 in saline prior to inoculation.

Minimum Inhibitory Concentration (MIC) Determination

MICs were determined by agar dilution using Mueller Hinton Agar for anaerobic organisms, Wilkens-Chalgren Anaerobe Agar for all anaerobes except the two Lactobacillus species, which were grown on DeMan Rogosa Sharpe Agar. A series of twofold dilutions of each essential oil (from 2.0-0.004 percent volume per volume [v/v]) was prepared and placed in sterile Petri dishes. Each dilution was placed into three Petri dishes and one of three agars was added to each plate and mixed thoroughly. Tween-20 (Sigma) was incorporated into the agar at a concentration of 0.5 percent (v/v) to enhance solubility. Clindamycin, neomycin, ampicillin, and ketoconazole (in doubling dilutions from 64 to 0.05 μg/mL) were used as positive controls, while dimethylsulfoxide (DMSO), Tween-20,
and plain agars were used as negative controls. Plates were dried at room temperature prior to inoculation.

Plates were inoculated with 1-2 μL spots containing approximately 10⁵ CFU for the anaerobic bacteria and 10⁴ CFU for the aerobic bacteria and *C. albicans* using a multipoint replicator (Mast Laboratories Ltd, Liverpool, UK). Aerobic organisms were incubated aerobically for 20-24 hours at 35°C; anaerobic organisms were incubated anaerobically for 48 hours at 35°C. Minimum inhibitory concentrations were determined after the incubation periods. The MIC was defined as the lowest concentration of essential oil that completely inhibited the growth of the organism in question. The presence of a single colony or a thin haze within the area of the inoculated spot was disregarded.

### Results

#### Minimum Inhibitory Concentrations

The MIC assay results of the nine essential oils are presented in Table 1. All essential oils tested displayed significant antimicrobial activity. The most potent essential oil was *Trachyspermum copticum*, which inhibited the growth of all microorganisms at a concentration of <2.2 percent. The most selectively acting oils were *Carum carvi*, *Lavandula angustifolia*, and *Trachyspermum copticum*, which inhibited the growth of potentially pathogenic *Bacteroides fragilis*, *Candida albicans*, and *Clostridium* spp., at concentrations that had no impact on either species of lactobacilli or bifidobacteria or the majority of other colonic organisms. *Citrus aurantium* var. *amara* displayed weaker antimicrobial effects, but was also selective in activity. The other oils were not selective in their activity. None of the negative controls (DMSO, Tween-20, and plain agar) had any impact on microbial growth.
Discussion

The antimicrobial properties of eight essential oils were evaluated against common members of the human gastrointestinal tract microflora. The essential oils were chosen based on the traditional uses of the herbs from which the essential oils are derived. For example, Mentha x piperita,22 Carum carvi,23 Foeniculum vulgare dulce,24 Mentha arvensis,25 Illicium verum,26 and Lavandula angustifolia27 have long been utilized as carminatives in Western herbal medicine. Citrus aurantium var. amara has a long history of use in traditional Chinese medicine for gastrointestinal antispasmodic and carminative activities,28 and Trachyspermum copticum29 have long been utilized as carminatives in Western herbal medicine. Citrus aurantium var. amara has a long history of use in traditional Chinese medicine for gastrointestinal antispasmodic and carminative activities,28 and Trachyspermum copticum29 have long been utilized in Ayurvedic medicine to relieve colic, flatulence, diarrhea, and dyspepsia.30

The most selectively acting oils were Carum carvi, Lavandula angustifolia, and Trachyspermum coticum, which at one concentration inhibited the growth of a number of potentially pathogenic microorganisms (Candida albicans, Clostridium spp., Bacteroides fragilis), while having no impact on the four species of beneficial microbes examined. Citrus aurantium var. amara essential oil was also selective in its activity. At concentrations that inhibited the growth of Bacteroides fragilis and Clostridium perfringens, no other species of bacteria or fungi was affected. Hence, these oils appear to have the most potential in the treatment of dysbiosis, where their use could help balance the GIT microflora.

IBS patients have been found to have lower fecal counts of lactobacilli, bifidobacteria, and coliform bacteria.24,25 The results of this study suggest that Carum carvi, Lavandula angustifolia, Trachyspermum coticum, and Citrus aurantium var. amara essential oils could be used in the treatment of IBS without negative ramifications on already disordered GIT microflora.

Other extracts were equally effective in killing both beneficial and potentially pathogenic members of the GIT flora, including Mentha x piperita, Foeniculum vulgare dulce, Mentha arvensis, and Illicium verum essential oils. Foeniculum vulgare dulce and Illicium verum essential oils were, however, less active toward lactobacilli than bifidobacteria or the potentially pathogenic organisms. Nonetheless, in concentrations that inhibited the growth of potentially pathogenic microbes, some beneficial bacteria were also inhibited.

Of these agents, only Mentha x piperita is commonly prescribed, due to its demonstrated efficacy in IBS.30–32 The study results suggest that the ingestion of M. x piperita essential oil may inhibit the growth of some common members of the GIT microflora. Until more research is conducted ascertaining the in vivo effects of M. x piperita essential oil on GIT microflora, it is prudent to prescribe a probiotic agent (containing both bifidobacteria and lactobacilli) concurrently with M. x piperita essential oil.

Generalization of these results to in vivo situations is limited, however, by the nature of the study design. It is unknown what impact the processes of digestion and absorption will have on an essential oil’s antimicrobial activity. Thus, the results of this in vitro experiment need to be interpreted cautiously and seen as solely preliminary. In vivo studies are needed to verify the selectivity of action displayed by these essential oils, as well as to address tolerability and safety concerns.

Future in vitro studies should take into account other common members of the GIT flora, such as Ruminococcus spp., Streptococcus spp., Peptococcus spp., Actinomyces spp., and Fusobacterium spp., as well as gas-producing microbes like methanogens and sulfate-reducing bacteria.33 The effects of carminatives on these latter two groups of bacteria would be particularly interesting. However, the results would still be preliminary and would not provide definitive evidence of in vivo effectiveness. Definitive answers await randomized, double-blind, placebo-controlled human trials utilizing the “gold standard” of microflora assessment techniques – 16S ribosomal RNA sequencing – to accurately delineate changes in the GIT microflora after ingestion of these essential oils.34

Conclusion

The most promising essential oils for the treatment of intestinal dysbiosis appear to be Carum carvi, Lavandula angustifolia, Trachyspermum coticum, and Citrus aurantium var. amara. The herbs from which these oils are derived have long been used in the treatment of gastrointestinal symptoms and these in vitro results suggest that their ingestion will have little detrimental impact on beneficial GIT microflora. More research is needed to investigate tolerability, safety concerns, and verification of selectivity.
References