Introduction

Elucidating the bioactivities of essential oils (EO) involves understanding interactions between multiple systems. For example, EO production is affected by changes in climate, soil conditions, allelopathic signals and endophytic associations. \(^{1-4}\) Furthermore, the subject’s physiological status as well as the method of application can influence the effectiveness. \(^{5-7}\) EO with a long ethnomedicinal history of use for improving mood and cognition include rosemary (Rosmarinus officinalis), lavender (Lavandula angustifolia) and sage (Salvia officinalis). \(^{5,8,9}\) Apart from the nootropic benefit of these plants, EO are being investigated as a therapeutic modality in Alzheimer’s disease and stress disorders. \(^{10-12}\) Naturalized apple mint (AM; Mentha suaveolens; syn. M. rotundifolia; syn. Mentha macrostachya Ten.; syn. Mentha insularis Req.) and native passionflower, Passiflora incarnata L, were selected for further investigation. Previous studies indicated that injected AM extracts exhibited depressant and neuromodulatory activity. \(^{13,14}\) Although the EO from the fruit of passionflower (PFF) have not been investigated, the stems and leaves are often used in herbal medicine and phytotherapy, as an anxiolytic, for reducing seizure incidence and for cessation of smoking. \(^{15,16}\) This study was conducted to determine if the aroma of EO from AM and PFF affect cognition and coordination and to assess the EO using GC-MS for components known to interact with olfactory receptors and neurotransmitters.

Methods

Plant materials were gathered from Seneca Creek Farm, Seneca, South Carolina. On separate days, whole fresh PFF or AM leaf was added to a food processor and blended for 90s. Then, the puree (~200 g) was added to a 5000 ml distillation flask. Approximately 2500 ml of dH2O was then added to the respective flasks and hydrodistilled for 2 hr on a Clevenger-type apparatus at a level sufficient for the water to boil and reflux. The essential oil was collected and separated from the hydolate. Participants for the cognition and coordination trial consisted of undergraduate volunteers (n=23) from Clemson University. The participants were not segregated by sex and were unaware as to the genuine aim of the study until the completion of testing. The trial was conducted in accordance with Clemson University guidelines. Each volunteer only participated in one trial (1 EO and 1 control) as to reduce software-familiarity bias. Participants were escorted to an isolated office in the Endocrine Physiology Laboratory and seated at desk with a computer where the cognition phase (CogLab, computer-assisted cognitive assessment software, Table 1) and the coordination phase (a bimanual game, Intercept\(^2\), Figure 1)
were conducted. The procedure for the cognition and coordination trial involved preparing an essential oil diffuser (Aura Cacia, Urbana, IA) by adding 40 µL of control oil (organic castor oil) to a cotton ball and then placing it in the diffusion chamber. Each participant was allotted a diffusion chamber and after an initial 5 minute familiarization period for Intercept², a screen capture program recorded game play for an additional 5 minutes at a rate of 1 capture per second. Subjects then completed an initial round of Memory Span, after which the data tables were saved. Participants then exited the trial room for 25 minutes while the diffusers were prepared with the variable essential oils (40 µL of AM or PFF). Another cycle of Intercept² and CogLab was completed by the participants while breathing in the aroma of AM or PFF EO. Results from the two cycles of Intercept² and CogLab were then compared and analyzed using Student’s T-Test in Excel and in GraphPad Prism. Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify the chemical components in AM and PFF EO. Fresh herbs were processed and extracted using hydrodistillation for approximately 2hr. Oils were separated from the hydrolat and stored at 4C. Multiple extractions were performed and combined for each herb. The samples were mixed for 5 minutes and then centrifuged for 10 minutes at 1200 g. The mixing and centrifuging was repeated for an additional 2ml of hexane; nonpolar layers were combined and refrigerated until GC-MS analysis. GC-MS analysis was performed on an Agilent 7890 GC with 5975 MS detector and utilized Wiley GC-MS library, Mass Transfer Library and Baser Library of Essential Compounds ¹⁸.

Results

For the cognition and coordination study, AM had a slight positive effect overall (ª=0.1), and PFF a significant reduction (ª=0.0005) on the scores in CogLab Memory Span, respectively (Figure 2, Table 2). The largest positive effect for AM (ª=0.05) was observed in the NM subtest. Conversely, PFF significantly reduced the scores on subtests on NM (ª=0.005) and LTS (ª=0.05). Conversely, in the coordination phase of testing, PFF had a slight positive effect (ª=0.1) and AM had no effect (Figure 3, Table 2). The GC-MS analysis identified the following chemicals in PFF EO: R-carvone >> Eucalyptol > Linalool (Figure 4). In AM EO, the chemicals constituents identified were: S-carvone >> Limonene > Dihydrocarveol > Terpinolene (Figure 5).

Discussion

In this study, the aroma of EO from AM slightly increased, and PFF decreased working memory, respectively. In the bimanual task, PFF enhanced and AM did
not affect skilled play. In the subtests of working memory, AM aided number recall, whereas PFF hindered recall of numbers and of letters that sound similar. These results may indicate that PFF and AM influence different pathways, as the discernment of letters differs from numbers at the neural level.

The chemicals identified by GC-MS analysis of PFF EO included R-carvone, eucalyptol and linalool. R-carvone has been reported to exhibit depressant and pain-relieving activity. Eucalyptol possesses multiple modes of action and linalool is known to act as an antidepressant and anxiolytic. The cumulative interactions of these components may have decreased adversely affected the working memory task.

The main chemical components revealed in the analysis of AM EO by GC-MS were S-carvone, limonene, dihydrocarveol, terpinolene and piperitone. S-carvone is capable of affecting autonomic arousal in humans. Carvone and limonene, like eucalyptol, can act through olfactory receptors and interactions with neurotransmitter systems. Limonene can modulate histamine and acetylcholine, perhaps to increase awareness. Exactly how the chemical components in PFF and AM affect neurotransmitter systems to influence memory and task performance is currently unknown, but may involve excitatory and inhibitory pathways.

References


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>LTD</td>
<td>Letters that sound different</td>
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<tr>
<td>LTS</td>
<td>Letters that sound similar</td>
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<td>LW</td>
<td>Long words</td>
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<td>NM</td>
<td>Numbers</td>
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<tr>
<td>SW</td>
<td>Short Words</td>
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Figure 1. Instructions for Intercept$^2$. 1) The “A,S,D,W” keys are to collect blue orbs with the blue paddle and the arrow keys are used to collect red orbs with the red paddle. 2) If the paddle touches the wrong color orb, points are lost and the paddle expands making it harder to maneuver. 3) If an orb falls to the ground, more points are lost and the area decreases. 4) Collect orbs to advance levels where the orbs fall faster and more frequently.
Figure 2.  a) Overall effect of Passionflower fruit (PFF) and Apple mint (AM) essential oils on CogLab. AM had a slight positive effect overall ($\alpha=0.1$) and PFF had a negative effect overall ($\alpha=0.0005$). b) AM had a positive effect on the NM subtest ($\alpha=0.05$) and PFF had negative effects on the NM ($\alpha=0.005$) and LTS subtests ($\alpha=0.05$).
Figure 3. Effect of PFF or AM EO compared to control EO on performance of Intercept². **PFF** had a slight positive effect on performance ($\alpha=0.1$).
<table>
<thead>
<tr>
<th>SubTest</th>
<th>Apple Mint vs Control</th>
<th>SD</th>
<th>Passionflower Fruit vs Control</th>
<th>SD</th>
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<tbody>
<tr>
<td>LTD</td>
<td>0.454</td>
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<td>0</td>
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<td>LW</td>
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<td>NM</td>
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<td>-1.167#</td>
<td>0.94</td>
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<th>Apple Mint</th>
<th>Passionflower Fruit</th>
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<tbody>
<tr>
<td>Overall</td>
<td>9.4</td>
<td>134.2</td>
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</table>

Note: AM (n = 11), * = significantly different than controls (α=0.05). Significance for PFF (n=12), ′ = 0.05; ″ = 0.005; † = 0.10.
Figure 4. GC-MS spectra for Passionflower Fruit essential oil.
Figure 5. GC-MS spectra for Apple Mint essential oil.