

Essential Oils from Apple Mint (*Mentha suaveolens*) and Passionflower Fruit (*Passiflora incarnata*): Studies on Cognition, Coordination, and Chemical Components

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Plant essential oils (EO) are used as perfumes, lotions and air fresheners because of their pleasant aromas, but EO also have the ability to elicit changes in mood and behavior. These activities are influenced by the mode of administration and by multiple signaling pathways. The EO aromas from organically grown apple mint (*Mentha suaveolens*) and passionflower fruit (*Passiflora incarnata*) were assessed for their effects on cognition and coordination. Participants completed two tasks designed to test working memory and bimanual task efficiency in rooms infused with apple mint, passionflower fruit or control EO. Bimanual coordination was assessed using the Intercept2 program and the Memory Span component of CogLab 2.0 was utilized as a test of working memory. Then, the EO were analyzed by GC-MS, resulting in the identification of several compounds with affinity towards olfactory receptors and neurotransmitter systems. For specific memory subtests within CogLab, EO from apple mint aided number recall, whereas passionflower fruit hindered recall of numbers and letters that sound similar. Passionflower fruit EO slightly enhanced bimanual task coordination. The results indicate that specific aromas may differentially affect task performance.

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 associations¹⁻⁴. Furthermore, the subject's physiological status as well as the method of application can influence the effectiveness⁵⁻⁷. 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 disorders¹⁰⁻¹². 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

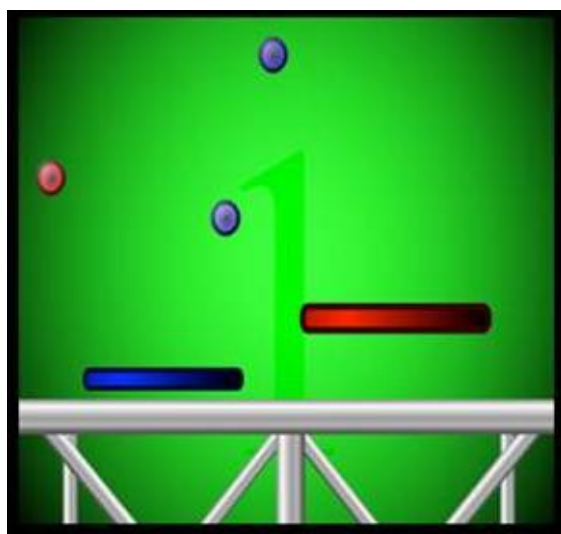
Plant materials were gathered from Seneca Creek Farm, Seneca, South Carolina. On separate days, whole fresh PFF or AM leaf was added to a food processor and blended for 90s. Then, the puree (~200 g) was added to a 5000 ml distillation flask. Approximately 2500 ml of dH2O was then added to

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 hydrolate. 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, Intercept², Figure 1) were conducted¹⁷.

Table 1. Subtest groupings from the memory span section of Cog Lab.

Abbreviation	Description
LTD	Letters that sound different
LTS	Letters that sound similar
LW	Long words
NM	Numbers
SW	Short Words

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



Intercept²

Figure 1. Instructions for Intercept². 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.

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 μ L 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 4°C. 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 Basler Library of Essential Compounds¹⁸.

Results

For the cognition and coordination study, AM had a slight

positive effect overall ($\alpha=0.1$), and PFF a significant reduction ($\alpha=0.0005$) on the scores in CogLab Memory Span, respectively (Figure 2, Table 2). The largest positive effect for AM ($\alpha=0.05$) was observed in the NM subtest. Conversely, PFF significantly reduced the scores on subtests on NM ($\alpha=0.005$) and LTS ($\alpha=0.05$). Conversely, in the coordination phase of testing, PFF had a slight positive effect ($\alpha=0.1$) 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^{19–22}. 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 activity¹⁹. Eucalyptol possesses multiple modes of action and linalool is known to act as an antidepressant and anxiolytic^{20,22,23}. The cumulative interactions of these components may have decreased adversely affected the working memory task^{22,24,25}.

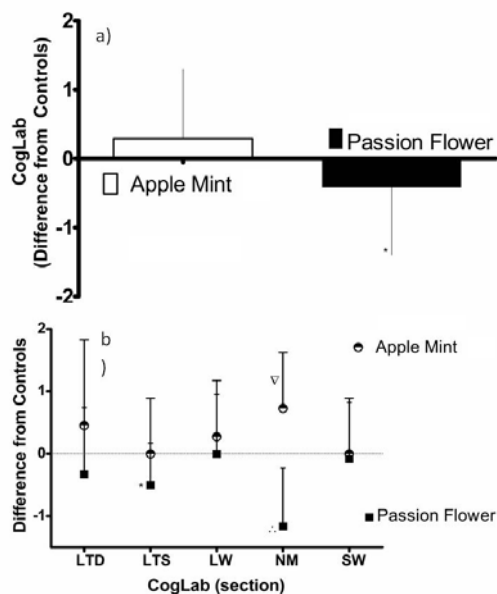


Figure 2. a) Overall effect of Passionflower fruit (PFF) and Apple mint (AM) essential oils on CogLab. AM had a slight positive effect overall ($\alpha=0.1$) and PFF had a negative effect overall ($\alpha=0.0005$). b) AM had a positive effect on the NM subtest ($\alpha=0.05$) and PFF had negative effects on the NM ($\alpha=0.005$) and LTS subtests ($\alpha=0.05$).

Table 2. Subtests of memory span component of CogLab and Intercept² scores for Passionflower Fruit and Apple Mint Essential Oils

SubTest	Apple Mint		Passionflower Fruit	
	vs Control	SD	vs Control	SD
LTD	0.454	1.37	- 0.333	1.07
LTS	0	0.89	- 0.500 ^v	0.67
LW	0.273	0.90	0	0.95
NM	0.727*	0.90	- 1.167 [#]	0.94
SW	0	0.89	- 0.083	0.90
Overall	0.291	1.01	- 0.416	0.98
Intercept ²	Apple Mint		Passion Flower Fruit	
Overall	9.4	134.2	137.5 [^]	303.0

Note: AM (n = 11), * = significantly different than controls ($\alpha=0.05$). Significance for PFF (n=12), v = 0.05; # = 0.005; ^ = 0.10.

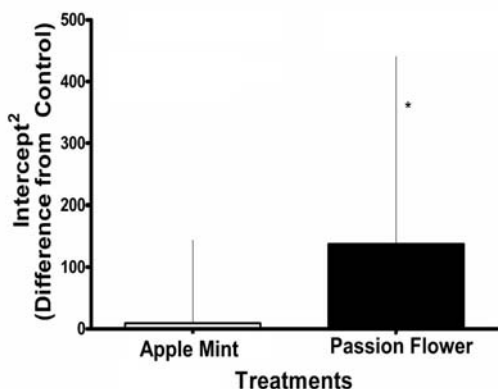


Figure 3. Effect of PFF or AM EO compared to control EO on performance of Intercept². PFF had a slight positive effect on performance ($\alpha=0.1$).

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^{26,27}. 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^{25,27–29}. 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.

Acknowledgements: This paper is dedicated to the memory of N. Dwight Camper, Professor of Plant Physiology at Clemson University and former President of the South Carolina Academy of Science.

Notes and references

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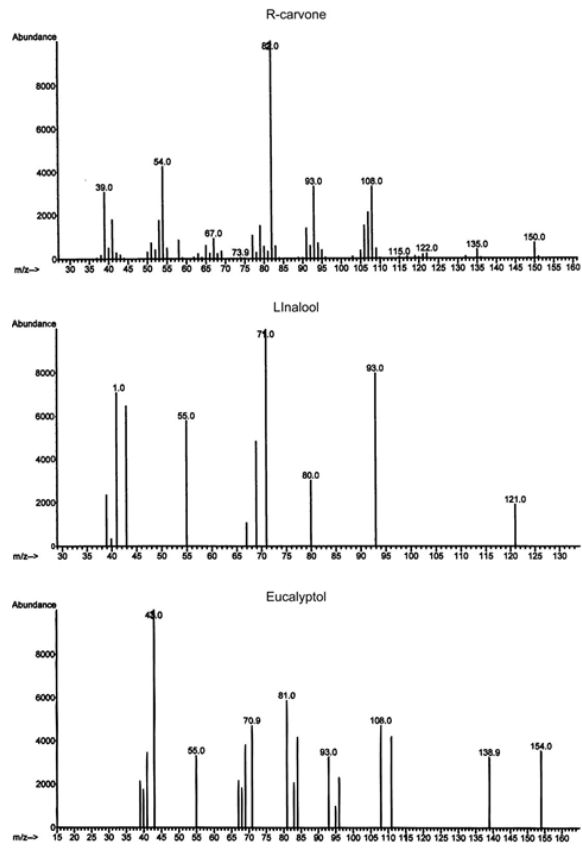


Figure 4. GC-MS spectra for Passionflower Fruit essential oil.

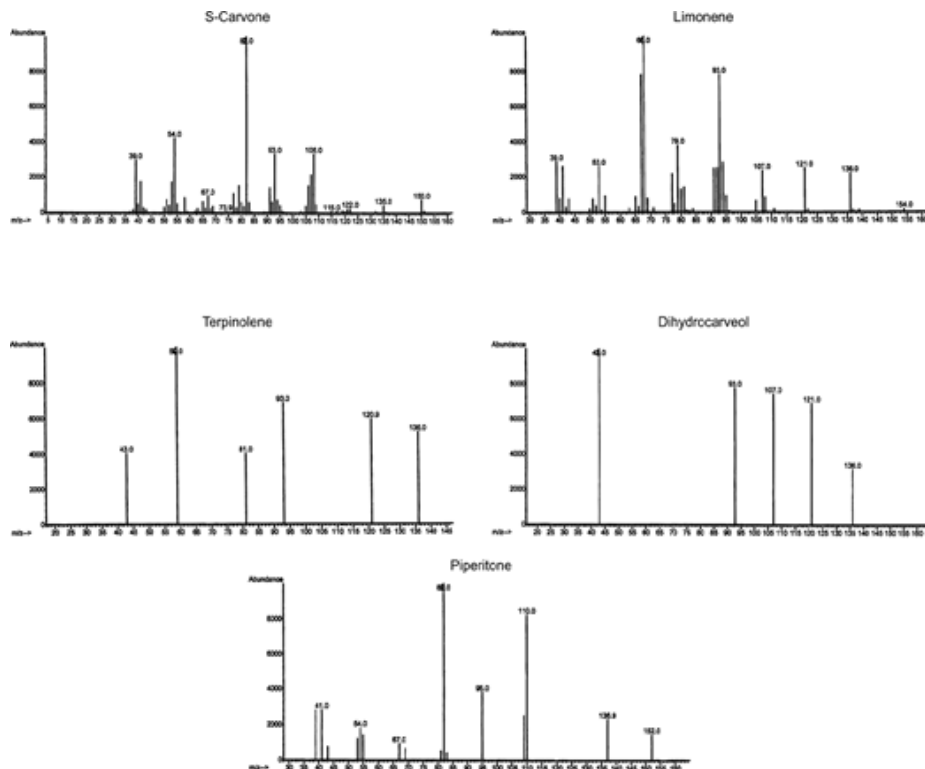


Figure 5. GC-MS spectra for Apple Mint essential oil.

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