Deciphering the Mysteries of Aromatic Incentive of Lemongrass Essential Oil Inhalation on Brain Activities in Healthy Adults

Israt Jahan¹, Esaba Sadia², Marefa Tuz Zohora Lima³, Farjana Boby⁴, Shaiful Islam Patwary⁵

¹Lecturer, Department of Physiology, Armed Forces Medical College
²Lecturer, Department of Physiology, Ibrahim Medical College
³,⁴Resident, Department of Physiology, Bangabandhu Sheikh Mujib Medical University
⁵Assistant Professor, Department of Physiology, Central Medical College

Abstract
With the rapid advancement in brain science and imaging technology, there is a growing interest in investigating the connection between aromatherapy and its effects on the brain. This study employs Quantitative Electroencephalography (QEEG) to examine the impacts of exposure to the fragrance of lemongrass essential oil (LEO) on specific brain regions. The objective is to assess the influence of inhaling the scent of LEO on EEG patterns in awake, healthy adult females using power spectral analysis. In this experimental study, 30 healthy adult female participants were exposed to a water mist as a control after baseline recordings. Subsequently, participants inhaled LEO aromatic mist diffused into the room air, and their brain activity was recorded. Data from specific scalp electrode readings were analyzed using the Wilcoxon Matched-Pairs Signed Rank Test. The results revealed a significant increase in the relative power of alpha waves in the prefrontal, frontal and occipital regions, accompanied by heightened theta power and decreased beta power. These results suggest that the inhalation of LEO oil modulates brainwave activities, inducing relaxing effects on the brain.

Keywords: Lemongrass essential oil, Quantitative Electroencephalography, Relative power

1. Introduction
Modern world has brought improvements in our quality of life and health care, thanks to evolving technologies and medical progress. However, it has also brought about increased mental pressures and higher expectations, which are affecting people's mental and emotional well-being.[1] Anxious mind can trigger feelings of depression, affecting people of all ages and genders in various ways. These effects can include difficulty in concentrating, changes in appetite, reduced brain function, and even potential neurological issues. In today's complex and competitive world, taking a break to relax can be beneficial for restoring energy levels amidst the hustle of daily life.[2]

Essential oils are a collection of plant-derived compounds, including various volatile chemicals, which collectively emit distinctive aromas, yielded from aromatic parts of the plants like leaves, flowers, seeds, barks, roots, fruits, peel or even the whole plant by distillation or by other physical processes.[3]
Aromatherapy, one of the most popular complementary therapies practiced globally, utilizes essential oils as main agents.[4] Among various application methods, inhaling fragrant oils is the most widely accepted form of aromatherapy, utilized to promote harmony in both mental and physical health.[1] Lemongrass (Cymbopogon citratus) is an aromatic perennial grass from the Poaceae family, also known as the sweet grass family. It serves as a popular plant-based remedy for common ailments in many countries and is prized for its culinary and perfumery uses due to its refreshing lemon-like aroma.[5] EEG measures the spontaneous electrical activity of the brain using electrodes placed on the scalp, providing insight into the collective neural activity of tens of thousands of neurons.[2] Essential oils' effects on the human neural activities have been showcased through QEEG recordings, aiding in the analysis of brain activity and behavior by examining the respective frequencies of signals in previous studies. It utilizes quantitative power spectral analysis by specific algorithm (most commonly Fast Fourier Transform), in order to reveal numerical parameters such as relative power, which expresses each frequency band as the percentage of total power of the signal in every single segment.[6]

The aim of the current study was to objectively detect effects in specific electrodes corresponding to various cognitive activities, utilizing delta (0.0-4.0 Hz), theta (4.0-8.0 Hz), alpha (8.0-13.0 Hz), and beta (13.0-25.0 Hz) brain waves as markers of aroma-induced neural modulation. Research on the effects of inhaling LEO is currently limited. Therefore, this study seeks to explore the impact of its inhalation on human brainwave activity.

2. Materials and methods
2.1. Ethical consideration: The study protocol, involving human subjects, followed the Helsinki (1964) ethical guidelines and obtained initial approval from the departmental ethical and academic committee, before receiving further approval from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University.

2.2. Participants
An advertisement for the study project was disseminated through social media and wall posters to recruit healthy adult female volunteers. Employing purposive sampling, a total of thirty female subjects were enrolled for the study to achieve a sufficient experimental sample size, with a significance level below 0.05 (Zα=1.96) and a test power of 80%.

2.3. Inclusion and exclusion criteria
The inclusion criteria involved, 1) 25 to 38 years old female, 2) right-handed, confirmed by using the Edinburgh Handedness Inventory scale, 3) normal sense of smell detected using 5) n-butyl alcohol test, and 4) individuals without symptoms of upper respiratory infection, hypertension, cardiovascular disease, neurological illness, or epilepsy, as well as 5) those who have no history of taking central nervous system medication, sedatives, or hormonal contraceptives currently or previously. Participants' assessment of the essential oil's pleasantness utilized a five-point Likert scale for odor familiarity, with individuals rating between 2 and 4 included in the study. Exclusion criteria encompassed participants allergic to the essential oil or experiencing headaches while inhaling.[7]

2.4. LEO collection and analysis
The essential oil was purchased from Sri Venkatesh Aromas, located in Delhi, India, a certified manufacturing company registered with The International Organization for Standardization. The company laboratory provided the GC/MS analysis report.
2.5. Experimental design
Initially, participants experienced a 'no aroma' session utilizing water mist as a control. Neural activity was measured using QEEG before and after this session. Following a break, participants were exposed to the aroma of LEO and recordings taken afterward. Mist was created by ultrasonic aroma diffuser which was placed on a table 30cm away from subject’s face.[8]

2.6. Participant preparation
Participants received a thorough explanation of the study's purpose, methods, procedures, and time requirements to ensure they could provide informed consent and comply with study instructions. They were advised to wash their hair, abstain from consuming stimulant-containing beverages like coffee, and to eat before testing to prevent fasting-induced EEG response alterations due to hypoglycemia. Also, participants were instructed to remove eyeglasses, earrings, and hairpins.[1]

2.7. Experimental procedure
Participants were seated comfortably in a soundproof room maintained at a controlled temperature (23-25°C), darkness, and humidity (40-50%). EEG recordings were conducted using the EEG (traveler) BrainTech 32+ CMEEG-01 (India), with 19 electrodes attached to the scalp according to the international 10-20 system and reference electrodes placed at the ear lobes (A1 and A2) using conductive paste. The recording settings included a gain of 7.5 µv/mm, 24-bit analogue-to-digital conversion, a notch filter at 50 Hz, a sampling rate of 1024 Hz, and impedance adjusted to 5 KOhm. High pass filter and low pass filter was set at 1 Hz and 35 Hz respectively. Baseline EEG measurements were recorded for 2 minutes, followed by a 20-minute inhalation of water mist, with EEG recording conducted for another 2 minutes. Subsequently, participants inhaled LEO mist for an additional 20 minutes, with EEG recording taken thereafter for an another 2 minutes. All recordings were conducted with eyes closed.

2.8. Data analysis
During each recording session, 60-second EEG waves from baseline and post-exposure periods were analyzed using BrainTech 40+ Standard version 4.47a software, which calculated the relative power of delta, theta, alpha and beta waves in FP1, FP2 (prefrontal), F3, F7, FZ, F4, F8 (frontal) and O1, O2 (occipital) leads. Each EEG recording lasted approximately 120 seconds, with 60 seconds of EEG waves selected for analysis. These 60-second data segments consisted of 15 epochs, each lasting 4 seconds and taken at 4-second intervals, excluding the first 10 seconds of data. Therefore, 15 frequency tables were generated, and the relative power of delta, theta, alpha, and beta waves from 9 electrodes in each of these frequency tables were averaged for each subject to compile the results. Normal distribution was checked by Shapiro-Wilk test. Data expressed as median and interquartile range (IQR), and the Wilcoxon Matched-Pairs Signed Ranks Test was used to compare pre-exposure and post-exposure values. Statistical analysis was conducted using SPSS version 24.0, with a p-value of < 0.05 considered statistically significant.

3. Results
3.1. Demographic characteristics of participants
Results of age, BMI, smell test and handedness score presented in Table 1. Data presented in mean and standard deviation (SD). Subjects were between 28 to 38 years of age (mean age 32.4 ± 2.50) with a body mass index ranged 20.5-24.92 kg/m² (mean BMI 23.35 ± 1.11); 1-butanol threshold test with a
score from 9 to 11 (mean score 10.3 ± 0.70) and Edinberg Handedness Inventory Scale with a score from 60 to 100 (mean score 73.33 ± 14.13).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28</td>
<td>38</td>
<td>32.4 ± 2.50</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.50</td>
<td>24.92</td>
<td>23.35 ± 1.11</td>
</tr>
<tr>
<td>Smell test (Bottle no.)</td>
<td>9</td>
<td>11</td>
<td>10.3 ± 0.70</td>
</tr>
<tr>
<td>Handedness (Score)</td>
<td>60</td>
<td>100</td>
<td>77.33 ± 14.13</td>
</tr>
</tbody>
</table>

### 3.2. EEG data
Following exposure to LEO, theta relative power exhibited contrasting activity, with an increase in the FP2 and F7 lead alongside with significant decrease in activity in the F4 lead area when compared to baseline and post-water mist recordings (Table 2). On the other hand, a notable increase in alpha power was observed in the FP2, F3, FZ, F4, O1, and O2 leads compared to baseline and post-water mist exposure (Table 3), accompanied by a significant decrease in beta wave activity across all prefrontal, frontal and occipital leads except O2 (Table 4).

<table>
<thead>
<tr>
<th>Leads</th>
<th>Baseline (B)</th>
<th>Post water mist exposure (W)</th>
<th>Post oil mist exposure (L)</th>
<th>p value B and W</th>
<th>p value B and L</th>
<th>p value W and L</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP1</td>
<td>10.9 (3.52)</td>
<td>11.3 (4.50)</td>
<td>13.9 (4.07)</td>
<td>0.198</td>
<td>0.046</td>
<td>0.229</td>
</tr>
<tr>
<td>FP2</td>
<td>10.7 (3.35)</td>
<td>9.70 (7.82)</td>
<td>14.2 (4.20)</td>
<td>0.478</td>
<td>&lt;0.0001*</td>
<td>0.002*</td>
</tr>
<tr>
<td>F7</td>
<td>13.6 (4.95)</td>
<td>13.1 (7.47)</td>
<td>16.3 (8.77)</td>
<td>0.837</td>
<td>0.008*</td>
<td>0.045*</td>
</tr>
<tr>
<td>F3</td>
<td>7.9 (5.17)</td>
<td>8.4 (5.57)</td>
<td>12.4 (4.77)</td>
<td>0.845</td>
<td>0.176</td>
<td>0.183</td>
</tr>
<tr>
<td>FZ</td>
<td>10.0 (5.0)</td>
<td>10.0 (8.0)</td>
<td>8.30 (5.60)</td>
<td>0.575</td>
<td>0.102</td>
<td>0.077</td>
</tr>
<tr>
<td>F4</td>
<td>13.2 (9.37)</td>
<td>12.6 (7.10)</td>
<td>7.7 (3.95)</td>
<td>0.178</td>
<td>&lt;0.0001*</td>
<td>0.004*</td>
</tr>
<tr>
<td>F8</td>
<td>13.3 (5.0)</td>
<td>11.7 (5.32)</td>
<td>14.70 (7.60)</td>
<td>0.381</td>
<td>0.440</td>
<td>0.052</td>
</tr>
<tr>
<td>O1</td>
<td>7.85 (3.55)</td>
<td>7.90 (6.90)</td>
<td>5.40 (4.08)</td>
<td>0.399</td>
<td>0.069</td>
<td>0.039</td>
</tr>
<tr>
<td>O2</td>
<td>10.90 (4.95)</td>
<td>7.90 (6.28)</td>
<td>9.50 (3.38)</td>
<td>0.136</td>
<td>0.360</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Conversely, there were no significant changes in delta activity in any of these leads, and also, post-water mist exposure did not result in detectable changes in brain waves. Among these data, which did not demonstrate pre-determined significance level when comparing post-LEO exposure with both baseline and post-water mist exposure, were not considered truly significant.

<table>
<thead>
<tr>
<th>Leads</th>
<th>Baseline (B)</th>
<th>Post water mist exposure (W)</th>
<th>Post oil mist exposure (L)</th>
<th>p value B and W</th>
<th>p value B and L</th>
<th>p value W and L</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP1</td>
<td>56.50 (16.75)</td>
<td>47.90 (28.20)</td>
<td>59.50 (14.80)</td>
<td>0.070</td>
<td>0.902</td>
<td>0.206</td>
</tr>
<tr>
<td>FP2</td>
<td>66.50 (14.62)</td>
<td>67.70 (23.30)</td>
<td>75.05 (11.35)</td>
<td>0.861</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>F7</td>
<td>65.7 (16.40)</td>
<td>62.50 (29.65)</td>
<td>59.40 (18.87)</td>
<td>0.159</td>
<td>0.040</td>
<td>0.781</td>
</tr>
</tbody>
</table>
4. Discussion

The responses in individual EEG leads triggered by LEO exposure were specifically analyzed to identify the types of cognitive functions it can influence. FP1 and FP2 electrodes placed over the prefrontal cortex, responsible for attention and logical reasoning; deviations in attention are indicated by the responses in the area of FP1, while FP2 responses are linked with reasoning and impulse control. F3 and F4 electrodes positioned over the frontal cortex, associated with motor planning; FZ is located near motivational centers, while F7 and F8 positioned in regions related to emotional processing and verbal expression.[2, 9] Theta waves (4.0 - 8.0 Hz) believed to reflect a relaxed mental state and creative ideation influenced by the subconscious, while also contributing significantly to short-term memory processing and the consolidation of memories.[10] It can be inferred that deep contemplation and emotional development may be promoted by inhaling LEO, while active cognitive processing is also reduced, indicating an overall relaxing effect on the cognitive network. This effect of increasing theta in this area also found after inhalation of Limnophilia aromatica, Chrysanthemum indicum and combined lavender and bergamot oil. Authors proposed a state of both physical and mental relaxation, characterized by a stable and comfortable condition linked to deeply internalized and tranquil physical states was achieved through aroma.[1, 2, 7] Significant alpha (8.0 - 13.0 Hz) activity in almost all leads represents a state where brain activities linked with relaxation, falling asleep, or resting, which does not necessarily halt active tasks; instead, such activities may be sustained or resumed. Similar findings were observed when subjects exposed to Limnophilia aromatica, Chrysanthemum indicum, Litsea cubeba, Cannabis sativa essential oil.[2, 7, 11] Authors claimed that boosting alpha wave activity heightens the sensation of calmness.

Beta waves (13.0 - 25.0 Hz) are linked to activities and actions and are present during conscious states like conversing, puzzle-solving, assessment, and decision-making.[9] Present
study showed lowered beta wave activity in all locations, which is similar to previous study.[2] The findings provide evidence that heightened theta and alpha wave activity, along with an overall decrement of beta wave, induce various forms of general relaxation and anxiolytic effects on the brain. Previous studies have shown the ability of LEO to induce a state of calmness. One study observed elevated theta and alpha power in the frontal area, indicating a deeply relaxed and positive mood.[12] Another study reported significantly improved task performance and a heightened sense of calm yet alert mood among their female participants.[13] According to GC/MS analysis, major component in LEO was citral (72.10%) in two isomers, cis-form (neral 31.46%) and trans-form (geranial 40.64%), which is similar to Litsea cubeba, Melissa officinalis and Verbena officinalis oil. Differences in the chemical configuration of compounds can influence odor perception and EEG responses, with each essential oil having its unique scent and specific effect on brain activity.[4] Fragnant molecules of essential oil inhaled through the nose, are conveyed via olfactory receptors to the limbic system, eliciting an emotional and reflexive response.[2] Various authors made several attempts to uncover the potential mechanism of citral as an anxiolytic through animal models, suggesting the involvement of the GABAergic pathway. Another study presented evidence indicating that enhancing both GABA and serotonin is essential to attain this tranquil state.[14, 15, 16]

5. Conclusion
This study has displayed the impact of inhaling LEO in conjunction with well-being and complementary practices. Lemongrass oil seems to influence brain function by inducing positive relaxation and anxiolytic effects. Incorporating this oil into a comprehensive integrated therapy targeting stress or depression relief is supported by the results of the study.

Limitations
One limitation of this study is that the findings may not be applicable to populations with diverse health conditions. Additionally, there is a possibility that the results could be influenced by psychological and environmental factors beyond the aroma presented. Furthermore, there was lacking of analytical software capable of dissecting the subdivisions within each frequency band (high, mid, low), which could have provided a more panoramic understanding of various cognitive functions.

Conflicts of interest
All authors declare that there are no conflicts of interest regarding the publication of this article.

Acknowledgement
Humble gratitude to all participants who generously contributed their time to this study, and to the research staff who provided invaluable assistance in data collection and analysis.

References


