We examined the effects of essential oil from legal (THC <0.2% w/v) hemp variety on the nervous system in 5 healthy volunteers. GC/EIMS and GC/FID analysis of the EO showed that the main components were myrcene and β-caryophyllene. The experiment consisted of measuring autonomic nervous system (ANS) parameters; evaluations of the mood state; and electroencephalography (EEG) recording before treatment, during treatment, and after hemp inhalation periods as compared with control conditions. The results revealed decreased diastolic blood pressure, increased heart rate, and significant increased skin temperature. The subjects described themselves as more energetic, relaxed, and calm. The analysis EEG showed a significant increase in the mean frequency of alpha (8–13 Hz) and significant decreased mean frequency and relative power of beta 2 (18.5–30 Hz) waves. Moreover, an increased power, relative power, and amplitude of theta (4–8 Hz) and alpha brain waves activities and an increment in the delta wave (0.5–4 Hz) power and relative power was recorded in the posterior region of the brain. These results suggest that the brain wave activity and ANS are affected by the inhalation of the EO of Cannabis sativa suggesting a neuromodular activity in cases of stress, depression, and anxiety.

1. Introduction

The European Pharmacopoeia, sixth Edition (2007), lists 28 essential oils (EOs) [1]. These oils are employed by inhalation and dermal (percutaneous absorption) and oral ingestion in the form of capsules. Industrial hemp (Cannabis sativa L. cultivars) is cultivated for fiber and seed production, but has an incredible number of possible applications as ingredient in the cosmetics industry, as flavouring for beverages (food industry), and in medicine. Several studies have been carried out on the cannabinoid content, resin, and seed oil of Cannabis sativa L. cultivars, but few studies have focused on the chemical composition and pharmacology of the essential oil extracted from fresh inflorescences and even fewer studies are concerned with its possible uses [2–6].

The hemp essential oil is a complex mixture of many volatile compounds, mainly monoterpenes, sesquiterpenes, and other terpenoid-like substances [7]. The main chemical components are myrcene, β-caryophyllene, limonene, α-pinene, β-pinene, terpinolene, and α-humulene. The general properties of these substances include antidepressant, relaxant, anxiolytic, sedative, antimicrobial, and antioxidants [8]. Some researchers studied the antibacterial properties of this oil. These findings show that industrial hemp EOs exhibited good antimicrobial activities, with respect to Gram (+) bacteria such as Enterococcus hirae, Enterococcus faecium, and S. salivarius subsp. thermophilus and against clostridia (in this case only C. sativa L. varieties Futura) [9]. The study of Russo describes pharmacological properties of main terpenoids present in industrial hemp EOs [8].
In the research by Bahia et al., they reported that \( \beta \)-caryophyllene may be useful in treating anxiety and depression. Moreover, they demonstrated the effect of \( \beta \)-caryophyllene and its underlying mechanism in a CB2 receptor-dependent manner in mice [10]. These \( \beta \)-caryophyllene's properties may explain why Cannabis users often cite relief of anxiety and depression as reason for their use. But, presently, the effects of hemp EOs inhalation on the brain in humans have not been studied and there are no studies on the possible therapeutic use. However, these studies support that hemp EOs inhalation can have a relaxing effect on the nervous system. Thus, this study is the first to focus on aspects such as brain wave activity and ANS parameters such as heart rate, blood pressure, respiratory rate, and skin temperature, as well as the assessment of mood states through comparative measures. Moreover gas chromatography characterization of hemp EOs was conducted.

2. Materials and Methods

2.1. Essential Oil Analysis. Hexane used for preparing working solution was purchased from Carlo Erba (Rodano, MI, Italy), while the linear n-hydrocarbons (C9–C40) were purchased from Sigma-Aldrich.

The EO used in this study is an extract of Cannabis sativa L. (Cannabaceae; hemp) purchased from Assocanapa Association (Carmagnola, TO, Italy). The EO was obtained from fresh leaves and inflorescences of Cannabis sativa L. were collected and steam distilled by Assocanapa Association, as given by the Italian Official Pharmacopoeia (2.5 L water distilled, 2 h in Clevenger-type apparatus). The Cannabis EO (CEO) yield was 0.11% v/w. CEO and EOs dilutions were stored at 4\(^\circ\)C before use. Gas chromatography/electronic ionization mass spectrometry (GC/EIMS) and gas chromatography/Flame Ionization Detector (GC/FID) analyses were accomplished using an HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 \( \mu \)m \( \times \) 0.25 mm, 0.25 \( \mu \)m film thickness), detector dual FID, working with the following temperature programme: 60\(^\circ\)C for 10 min, ramp of 5\(^\circ\)C/min to 220\(^\circ\)C; injector and detector temperatures 250\(^\circ\)C; carrier gas nitrogen (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 \( \mu \)l. For both columns, components were identified by comparing their retention times with those of pure authentic samples, comparing their l.r.i. relative to the series of n-hydrocarbons (C9–C40), and on computer matching against commercial (NIST 98 and ADAMS 95) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data [13, 14].

The percentage compositions were computed from the GC peak areas. Moreover, the molecular weights of the all identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas. Analysis of the essential oils identified 35 constituents (Table 1), accounting for 97.6% of the total oils (only compounds >0.1% are reported in Table 1). Monoterpenic hydrocarbons represented the 57.2% of total volatiles and myrcene was the main constituent (22.9%). Sesquiterpenes hydrocarbons represented the second class of volatiles (34.3%) with the most abundant being \( \beta \)-caryophyllene (18.7%).

2.2. Subjects. Five healthy volunteers (3 males and 2 females) aged 30 to 57 years (mean age 40.8 ± 12.19 years) and with body mass index (BMI) between 19.05 and 34.60 kg/m\(^2\) (mean BMI 26.986 ± 7.18 kg/m\(^2\)) participated in this study. Demographic data of the participants is presented in Table 2. Only five volunteers were available for the experimental session preprogrammed; other recording experimental sessions were not made because environmental parameters would not be reproducible and comparable. None of the subjects had cardiovascular disease, they did not exhibit any symptoms of upper respiratory infection, and women were not to be in their menstrual period on the day of the tests. Two subjects were smokers and one of the male subjects had a slight headache at the time of the experiment. All experimental procedures were followed with the strict ethical standards formulated in the Helsinki Declaration of 1964 that was revised in 2000 and all the subjects have participated in the study after signing the informed consent. The study was conducted in private healthcare facilities outside the network of the Regional Health System. Therefore, any ethical and managerial assumption is rooted in the direct relationship between the patient, who has released the relative consent, and the host structure.

2.3. Methods. One milliliter of sweet almond oil (SAO, base oil, purchased from Carlo Erba) was used for control condition as placebo and one milliliter of CEO was administered according to the protocol described in Figure 1. The sweet almond oil was administered with gauze and participants were asked to inhale simultaneously with both nostrils. The same procedure was also adopted for the CEO. In accordance with previous studies, it has been found that the pleasantness of the aroma of the oil could alter autonomic activity [15, 16]. As a result of these facts, the subjects were asked to inhale SAO and CEO to assess the pleasantness or less pleasantness of the aroma.

2.4. ANS and Mood State Measurement. The mood state and ANS parameters of the subjects such as blood pressure, heart rate, skin temperature, and respiratory rate were recorded simultaneously. The ANS parameters were measured manually. While the evaluation of the changes of moods was made...
through a subjective evaluation of the participants using a list of 8 terms selected for their relevance to describe affective feelings induced by odors and mood state after inhalation of CEO: anxiety, calm, hungry, hilarity, fatigue, apathy, energy, and heavy eye [17–19].

2.5. EEG Recording. A set of 21 electrodes with an additional ground electrode and a reference electrode were placed onto the subject's head with electrocap in accordance with the international 10–20 system at F1, F2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T3, P3, Pz, P4, T6, O1, and O2. Mizar 40 EB Neuro-Firenze was used as the recording system. Sampling rate was set at 512 Hz; the HF filter was set at 70 Hz; time constant 0.3; range –/+/4.1 mV. The relative power spectrum of the respective frequency bands was expressed as follows: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–14 Hz), and beta (14–30 Hz). Furthermore, the beta wave was further categorized as beta 1 or low frequency beta (14–18 Hz) and beta 2 or high frequency beta (18.5–30 Hz) activities.

2.6. Experimental Protocol/Procedure. All the steps in this experiment were similarly conducted as the previous study recorded on the effects of rosemary oil inhalation [20]. All activities were conducted in a quiet room and the subjects were seated in a comfortable chair. The ANS electrodes were attached to the appropriate positions. The ANS parameters: heart rate, skin temperature, respiratory rate, and systolic and diastolic blood pressure were recorded at the beginning of the experiment before resting (baseline) EEG measurement and after CEO inhalation.

The experiment consisted of three trials: first session served as a baseline (resting period) and took ten minutes. The second and the third sessions took five minutes each. The first session baseline EEG recording was done with both eyes opened and eyes closed, respectively. In the second session, SAO was inhaled by the subjects. In the third session, CEO was applied and mood state was measured after inhalation. EEG was recorded for five minutes during SAO inhalation and after five minutes of rest it was recorded again for five minutes during CEO inhalation. After the recordings, the subjects were asked to give their preference and impression of the odors presented and of their moods (Figure 1).

2.7. Statistical Analyses. The MedCalc statistics for biomedical research software version 16.2.1 was done for data analysis of the effects of CEO on physiological reactions and mood states, before and after the hemp inhalation. A nonparametric Kruskal Wallis signed rank test was used for EEG data analysis and Friedman test was performed to determine whether the activity changed significantly in any of frequency bands in P4-O2 and P3-O1 brain region. A paired t-test was carried out on data concerning the subjects' blood pressures, heart rates, skin temperatures, and respiratory rates. A p value < 0.05 was considered significant. A percentage evaluation was done for the mood states.

3. Results and Discussion

In the present research, CEO was administered by inhalation to healthy subjects and we examined the effects of the oil on the human nervous system. Brain wave activity and ANS parameters (blood pressure, heart rate, respiratory rate, and skin temperature) were recorded as the indicators of the arousal level of the nervous system. In addition, we studied the effects of CEO on moods by performing subjective self-evaluation in order to assess the arousal levels.
Myrcene, the main component of CEO, has a sedative, analgesic, and relaxing activity [23, 24]. Thus, these results confirm that CEO contains mood-elevating bioactive components that prove to be beneficial to its users.

3.3. EEG Parameters. EEG spectral analysis was done with quantitative evaluation of windows on 2 seconds with Interpolation Algorithm Rectangular.

Five frequency bands were evaluated (delta, theta, alpha, beta 1, and beta 2) and values of power (µV²), amplitude (µV), relative power (µV²), and mean frequency were calculated during rest, during SAO inhalation, and during CEO inhalation states. The studied areas were divided into the right posterior area (P4-O2) and left posterior area (P3-O1) brain regions. The data recording shows an alteration of EEG during exposure to the CEO. There were noticeable changes of band power, amplitude, and relative power in alpha, theta, delta, and beta waves as reported in Tables 5, 6, 7, and 8 (mean and median value).

During the CEO inhalation the power, the relative power, and amplitude of the alpha waves in both brain regions were increased and mean frequency for alpha significantly increased (compared with SAO) in P4-O2 brain area (p < 0,05). The power and relative power changes in the theta and delta waves in the left posterior brain region were also increased. In contrast, the power in the delta wave in the right posterior brain region was decreased. A significant decrease was observed in the case of the beta 2 wave's relative power (compared with resting condition) and mean frequency (compared with SAO) in P4-O2 (p < 0,05), Figures 2 and 3. The present research shows the effects of CEO inhalation on brain waves. This research showed that alpha (8–13 Hz) and theta (4–8 Hz) activity increased during CEO exposure in the posterior regions, and mostly left posterior area P3-O1 brain regions. Moreover, alpha mean frequency increased significantly in P4-O2 region. These results show

**Table 2: Demographic data for subjects.**

| Parameters | Subjects (*M, *F) | Minimum | Maximum | Mean | SD |
|------------|-----------------|---------|---------|------|----|
| Age 57     | M1 30 M2 50 M3 30 F1 37 F2 30 | 57      | 40,8    | 12,19|
| Weight (Kg) 90 | M1 100 M2 95 M3 55 F1 50 F2 50 | 100     | 78      | 24,04|
| Height (cm) 176 | M1 170 M2 95 M3 165 F1 162 F2 162 | 176     | 169     | 5,56 |
| BMI (Kg/m²) 29,05 | M1 34,6 M2 32,11 M3 20,2 F1 19,05 F2 19,05 | 34,6    | 26,99   | 7,18 |

* M: Male; * F: Female.
Table 3: In detail the values, mean, and standard deviation of ANS parameters for resting condition and after Cannabis oil inhalation.

| Subjects (M, F) | Demographic data     | ANS parameters                      | Cannabis essential oil                      | Skin temperature (°C) |
|----------------|----------------------|-------------------------------------|--------------------------------------------|-----------------------|
|                | Age                  | Weight (kg) | Height (cm) | BMI (kg/m) | Systolic blood pressure (mmHg) | Diastolic blood pressure (mmHg) | Heart rate (bmp) | Respiratory rate (bpm) | Systolic blood pressure (mmHg) | Diastolic blood pressure (mmHg) | Heart rate (bmp) | Respiratory rate (bpm) | Skin temperature (°C) |
| M1             | 57                   | 90         | 176         | 29,5       | 140                    | 90                        | 88               | 14                     | 36,5                | 150                    | 95               | 88               | 13                | 36,5               |
| M2             | 30                   | 100        | 170         | 34,6       | 120                    | 80                        | 66               | 18                     | 36,3                | 115                    | 75               | 74               | 14                | 36,7               |
| F1             | 30                   | 55         | 165         | 32,11      | 88                     | 65                        | 72               | 14                     | 36,6                | 85                     | 60               | 72               | 15                | 36,8               |
| M3             | 50                   | 95         | 172         | 32,11      | 125                    | 95                        | 62               | 15                     | 36,2                | 120                    | 80               | 75               | 15                | 36,4               |
| F2             | 37                   | 50         | 162         | 19,0       | 110                    | 70                        | 88               | 18                     | 36                  | 108                    | 65               | 94               | 20                | 36,4               |
| MEAN           | 40,8                 | 78         | 169         | 26,9       | 116,6                  | 80                        | 75,2             | 15,8                    | 36,3                | 115,6                  | 75               | 80,6            | 15,4            | 35,6               |
| SD (±)         | 12,2                 | 24,0       | 5,6         | 7,2        | 19,3                   | 12,7                      | 12,2             | 2,1                     | 0,24                 | 23,4                    | 13,7             | 9,8              | 2,7              | 0,18               |

*P < 0.05 significance when compared to resting condition; *M: Male; *F: Female.
Table 4: Percentages of emotional states scores after cannabis essential oil inhalation.

| Subjects (*M, *F) | Age | Weight (kg) | Height (cm) | BMI (kg/m) | Anxiety | Calm | Hunger | Hilarity | Heaviness eye | Tiredness | Apathy | Energy |
|-------------------|-----|-------------|-------------|------------|---------|------|--------|----------|-------------|-----------|--------|--------|
| M1                | 57  | 90          | 176         | 29.5       | Decreased | -    | Increased | -        | -           | Increased | -      | Increased |
| M2                | 30  | 100         | 170         | 34.6       | Decreased | Increased | Increased | -        | -           | Increased | -      | Decreased |
| F1                | 30  | 55          | 165         | 20.2       | -        | Increased | Increased | -        | -           | Increased | -      | Decreased |
| M3                | 50  | 95          | 172         | 32.1       | -        | Increased | Increased | -        | -           | Increased | -      | Decreased |
| F2                | 37  | 50          | 162         | 19.0       | -        | Increased | -         | Increased | -           | -         | -      | Increased |
| %                 | 40% | 80%         | 80%         | 40%        | 60%      | -    | 40%     | 60%/40%  |

*M: Male; *F: Female.
Table 5: Mean and median power values and $p$ value for resting condition, sweet almond oil inhalation, and cannabis essential oil inhalation.

| Brain area | Resting | Sweet almond oil | Cannabis essential oil | $p$ value |
|------------|---------|------------------|------------------------|-----------|
| Theta power ($\mu V^2$) |         |                  |                        |           |
| P4-O2      | 3,286 (2.77) | 3,704 (2.75)     | 3,366 (3.36)           | 0.268     |
| P3-O1      | 3,166 (3.19) | 3,522 (3.16)     | 7,776 (5.21)           | 0.497     |
| Alpha power ($\mu V^2$) |         |                  |                        |           |
| P4-O2      | 41,266 (38.95) | 48,592 (39.41)   | 58,788 (40.17)         | 0.599     |
| P3-O1      | 46,698 (48.45) | 36,8 (30.88)     | 51,036 (51.26)         | 0.268     |
| Delta power ($\mu V^2$) |         |                  |                        |           |
| P4-O2      | 10,734 (4.68) | 18,96 (12.23)    | 15,36 (15.72)          | 0.167     |
| P3-O1      | 8,768 (8.4)   | 13,756 (8.14)    | 19,542 (17.09)         | 0.268     |
| Beta 1 power ($\mu V^2$) |         |                  |                        |           |
| P4-O2      | 5,816 (4.61)  | 8,778 (5.53)     | 9,05 (7.12)            | 0.849     |
| P3-O1      | 6,402 (8.19)  | 8,01 (7.61)      | 8,268 (8.4)            | 0.849     |
| Beta 2 power ($\mu V^2$) |         |                  |                        |           |
| P4-O2      | 3,086 (4.05)  | 2.47 (3.08)      | 2.83 (3.1)             | 0.268     |
| P3-O1      | 2,714 (2.64)  | 2.918 (2.3)      | 3.734 (4.14)           | 0.849     |

SAO: sweet almond oil; CEO: cannabis essential oil.

Table 6: Mean and median relative power values and $p$ value for resting condition, sweet almond oil inhalation, and cannabis essential oil inhalation.

| Brain area | Resting | Sweet almond oil | Cannabis essential oil | $p$ value |
|------------|---------|------------------|------------------------|-----------|
| Theta PotR |         |                  |                        |           |
| P4-O2      | 5,082 (4.06) | 5,048 (4.61)     | 4,642 (3.78)           | 0.268     |
| P3-O1      | 5,01 (5.53)  | 5,386 (5.67)     | 7,248 (6.32)           | 0.497     |
| Alpha PotR |         |                  |                        |           |
| P4-O2      | 65,098 (69.39) | 58,608 (60.74)   | 60,042 (63.23)         | 0.497     |
| P3-O1      | 67,488 (68.22) | 55,212 (56.46)   | 55,746 (50)            | 0.268     |
| Delta PotR |         |                  |                        |           |
| P4-O2      | 15,64 (14.32) | 22,254 (24.25)   | 21,284 (25.16)         | 0.497     |
| P3-O1      | 13,234 (13.36) | 21,606 (20.48)   | 23,34 (25.39)          | 0.497     |
| Beta 1 PotR |         |                  |                        |           |
| P4-O2      | 8,224 (8.4)   | 9,444 (7.98)     | 9,09 (9.89)            | 1.00      |
| P3-O1      | 9,106 (7.57)  | 12,054 (8.59)    | 8,318 (9.4)            | 0.849     |
| Beta 2 PotR |         |                  |                        |           |
| P4-O2      | 4,482 (2.97)  | 3,028 (2.82)     | 3,432 (2.48)           | 0.05308*  |
| P3-O1      | 3,986 (2.84)  | 4,226 (3.89)     | 3,862 (3.54)           | 0.849     |

SAO: sweet almond oil; CEO: cannabis essential oil. *CEO differs from resting with $p$ value < 0.05.

Concordance with the past EEG studies on the effects of odors which demonstrated increased alpha activity by administration of several EOs such as lavender, chamomile, $\alpha$-pinene, and limonene oil [25–28]. Instead alpha activity is attenuated under emotional tension and stress condition [29].

The EEG evidence of relaxation can be seen in various practices such as meditation, yoga, Qigong, and mindfulness [30, 31]. The study among people meditating can demonstrate similar EEG changes with CEO inhalation, which presented as increase in theta and alpha activities in the brain during meditation [32, 33]. In addition, the studies of Aftanas [34–37] show that during meditation there is also release of hormones such as melatonin, serotonin, and cortisol. These results lend support that increases in theta and alpha waves activity cause a range of general relaxation and anxiolytic effects on the brain and also some possible decreases of pain. Thus, the data recorded after CEO inhalation shows relaxation and anxiolytic effects on the brain at level of the ANS, CNS, and mood states. At level of mood states a feeling of calm, relaxation, and decreased anxiety was recorded indicating the involvement of the limbic system.

The changes in ANS parameters (heart frequency, skin temperature, and diastolic blood pressure) can be explained by the $\alpha$-pinene activity on the parasympathetic system...
and limonene activity on the sympathetic system action. Komiya et al. [22] found that limonene increases serotonin in the prefrontal cortex, and dopamine (DA) in hippocampus mediated via 5-HT1A. This determines the direct activation of the sympathetic system. At levels of CNS activity, alpha and theta waves increased indicating a relaxing effect and antidepressant and antianxiety effect due to the β-caryophyllene and limonene. The analgesic action of CEO on the subject with headache may be explained by increase of alpha and theta waves and abundance of terpenes such as myrcene, limonene, and β-caryophyllene.

4. Conclusions

The small study population is a limitation of this study but it is however a preliminary study. Further studies of the effect of CEO on the brain are needed with a wider sample in order to have a greater number of significant data. However, the
Figure 2: Each panel shows the mean of power, relative power, mean frequency, and amplitude values of alpha and theta activities for the resting and sweet almond oil (SAO) and cannabis essential oil (CEO) inhalation states. The theta and delta waves increased mostly in P3-O1.

Results lend some support for including CEO in a perspective integrated therapy aimed at relieving stress or depression.

The results suggest the occurrence of the positive relaxation and anxiolytic effects of CEO. These findings provide evidence that brain wave activity autonomic nervous system response and mood states were affected by CEO.

**Abbreviations**

ANS: Autonomic nervous system
CNS: Central nervous system
CB2: Cannabinoid receptor type 2
EEG: Electroencephalography
EO: Essential oil
Figure 3: Values of mean frequency and power relative to alpha and beta 2 with significant difference in P4-O2 area of brain.

CEO: Cannabis essential oil
SAO: Sweet almond oil
GC/FID: Flame Ionization Detector
GC/EIMS: Gas chromatography electronic ionization mass spectrometry
PNS: Parasympathetic nervous system
THC: Tetrahydrocannabinol.

Conflicts of Interest

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

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Evidence-Based Complementary and Alternative Medicine

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