Universitat Autònoma de Barcelona

Effects of Aromatherapy on negative emotions and work and study performance: Heart Rate Variability analysis to assess the balance of the Autonomic Nervous System

Lin Huang

Doctoral Thesis

Effects of Aromatherapy on negative emotions and work and study performance: Heart Rate Variability analysis to assess the balance of the Autonomic Nervous System.

Lin Huang

Thesis Director: Dr. Lluís Capdevila Ortís

PhD Program in Health and Sport Psychology
(Doctorat en *Psicologia de la Salut i de l'Esport*)
Departament de Psicologia Bàsica, Evolutiva i de l'Educació
Facultat de Psicologia
Universitat Autònoma de Barcelona

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ABBREVIATIONS

AG: Aromatherapy group

ANS: Autonomic nervous system

AR: Aromatherapy

BTv4: Bluetooth v4

CAB: Citrus aurantium bigarade

CG: control group

CHM: Chinese herbal medicine

CL: Canarium luzonicum (langxiangzhi)

CO: Cananga odorata(ylang)

CON: control group

DIF: different

Dur: during

EXP: experiment group.

FACE: Feeling Scale

FIG: Figure

GLM: general linear model

HF: high frequency

HFnu: HF values expressed in normalized units

HRV: Heart rate variability

HR: heart rate

LF: low frequency

LFnu: LF values expressed in normalized units

M: mean

MCS- Mental Component (SF12)

Min: minter

N: number

NS: no significance

OBL: Ocimum basilicum, L

OFC: orbitofrontal cortex

PAV: Prunus amygdalus var

PCS- Physical Componente (SF12)

P: statistical significance level

PRE: previous period

POMS: Profile of Mood States

POST: after inhalation period

RMSSD: root mean square of differences of successive RR intervals

RRmean: the mean of RR intervals

SD: standard deviation

SDNN: standard deviation of all RR intervals

Ses: session

SF-12 : The 12-item Short Form Health Survey

STAI: The State-Trait Anxiety Inventory (STAI)

STAI-S: State scale of measures State- Anxiety Inventory

STAI-T: State scale of State-Trait Anxiety Inventory

TCM: Traditional Chinese medicine

VLF: very low frequency band

WHO: World Health Organization

PROLOGUE

My name is Lin Huang and I arrived from China in 2013 with the intention to course a PhD program in Health Psychology during four years. Currently, I am an associated professor in Kunming University (China) and my teaching subject is *Leisure Tourism Management* and *Health Tourism Management*. All along, I thought about how to improve my professional knowledge. I have always been interested in applying healthy lifestyle in the professional environment. Thus, I studied Traditional Chinese Medicine (TCM) in Yunnan Traditional Chinese Medicine University for five years. TCM uses good methods to reduce negative emotions, but I realized I wanted to learn from the medical psychology of the western world as well. This is the reason why I came to Europe.

I knew the PhD program in Health Psychology of the Autonomous University of Barcelona (UAB) has acquired considerable experience and recognition abroad. I considered it was a great opportunity for me to study at UAB with the research group "Health, Sport and Lifestyle" coordinated by Dr Lluis Capdevila. Here I could not only study about healthy lifestyle and physical activity, but I would also learn many aspects about Catalonia and Spain as ancient and rich historic countries in Europe: the tourism industry, which is highly developed; Spanish people, who love sports, who are cheerful, have a great sense of humour and are keen and enthusiastic; and the healthy Mediterranean diet. I was so excited with this perspective, when I received the offer from the Health, Sport and Lifestyle research group. If we combine the experience of TCM treatment and the European empirical research and we are open to learn from each other, what kind of interesting new findings will come to light?

The studies and the subject of this thesis, titled *"Effects of Aromatherapy on*

negative emotions and work and study performance: Heart Rate Variability analysis to assess the balance of the Autonomic Nervous System", is part of the research lines of the group "Health, Sport and Lifestyle" (2014SGR-1497, Generalitat de Catalunya). In particular, the thesis is part of the objectives of the two research projects:

a) "Real-time psycho-physiological analysis system using mobile devises to manage stress and effort when doing physical activity" (DEP2015-68538-C2-1-R; Ministerio de Economía y Competitividad, Spanish Government; 2016-2018).

b) "HRV and cognitive-behavioural parameters as individual differences markers in an intervention to improve healthy lifestyle" (PSI 2011-29807-C02-01/PSIC; Ministerio de Ciencia e Innovación, Spanish Government; 2012-2015)

We divided the thesis in two general parts. The first one (Theoretical Part) is the background of Aromatherapy in the main context of Traditional Chinese Medicine. We will also take a look at the effects of Aromatherapy over physical and psychological symptomatology. The second part (Empirical Part) is dedicated to analyse the aromatherapy efficacy for improving negative emotions, attention level and performance in the workplace and in academic situations. To achieve this general objective we will conduct two main studies (Study 1 and Study 2) with their respective pilot-studies (Pilot-Study 1 and Pilot-Study 2):

1) The Pilot-Study 1 is planned to obtain the key information to design the definitive Study 1, such as the type of essential oil, the number of sessions or the inhalation time duration. The main objective of Study 1 is to analyse the short and long term efficacy of aromatherapy for reducing anxiety and improving study concentration of university students. For this reason, the Pilot-study 1 will be conducted on university students in order to achieve the best results.

2) In the same way, the Pilot-Study 2 is planned to obtain the key information to design the final Study 2 carried out with university staff. The main purpose of Study 2 is to analyse the AR efficacy to reduce workplace stress and improve work performance in university administrative workers. The results of Study 2 have already been published in the paper that you can find in the annexes part:

Huang L and Capdevila L. (2016). Aromatherapy Improves Work Performance Through Balancing the Autonomic Nervous System. *The Journal of Alternative and Complementary Medicine.* Ocober 2016, pp. 1-8. DOI: 10.1089/acm.2016.0061

In all studies and pilot-studies we will analyse the AR effects over the heart rate variability (HRV) as a marker of the sympathetic/parasympathetic balance from the ANS. For this purpose, we will use the FitLab® Team system (Health&SportLab, Barcelona, Spain).

SUMMARY

The general aim of this thesis is to analyse the aromatherapy (AR) efficacy to reduce negative emotions and increase the attention level and performance in the workplace and in academic situations. Its efficacy will be tested through questionnaires and by checking the balance of the Autonomic Nervous System through HRV analysis. To carry out this general objective, we conducted two main studies. We will analyse the AR effects over the heart rate variability (HRV) as a marker of the sympathetic/parasympathetic balance from the ANS.

The objective for Study 1 was to analyse the AR efficacy for reducing anxiety and improving study concentration in university students. The sample was composed by 12 students (age between 18 and 33 years old). We analysed the effects of *basil* essential oil inhalation at short-time (1 hour) and at long-time (1 month). We found that Inhalation of *Basil* essential oil reduces anxiety scoring and improves cardiac variability (SDRR and RMSSD parameters). AR in a short session or with an aromatic necklace every day during one month produces emotional and HRV changes on participants.

The objective for Study 2 was to analyse the aromatherapy efficacy to improve the work performance and reduce the workplace stress. The sample was composed by 42 administrative university workers (age: 42.21 years, SD=7.12; gender: 10 men and 32 women). All sessions were performed in a university computer classroom. The participants were randomly assigned into an aromatherapy group (AG) and a control group (CG), and they were invited to participate in a specific session only once. They were seated in front of a computer. During the intervention period, some oil diffusers were launched and worked throughout the session with petit grain essential oil for AG sessions and a neutral oil (almond) for CG sessions. At the same time, participants completed a computer task in a specific website typing on their keyboard until they finished it. The single times were different for all participants and were recorded in the website as “Performance Time”. The aromatherapy group performed the website task with the keyboard 2, 28 min faster than CG typing ($p=0.05$) and showed better cardiac variability (HF, SDNN and RMSSD parameters). Inhalation of *Petitgrain* essential oil reduces stress and improves performance in the workplace. AR improves the

performance time when typing with the keyboard in a simulated computer work session and produces some changes improving HRV.

General Conclusions: This doctoral thesis confirms that aromatherapy (inhaling essential oils) can improve performance in the workplace, can help to manage negative emotions and can increment attention level in academic and workplace situations. Our results confirm that inhalation of essential oils produces this effect by balancing the sympathetic/parasympathetic system of the Autonomic Nervous System. HRV analysis is a good marker of this effect. This ANS balance can be explained by a combined action of the *Petitgrain* and *Basil* main components. Some of them have sedative effects, which can induce changes on the parasympathetic activity, and other components have activation effects, which stimulate the sympathetic activity. The final effect can be an improvement of the mental and emotional condition by a combination of reducing stress level and increasing arousal level of the participants in terms of attentiveness and alertness. We can also explain our results according to TCM. Essential oils can work through a truly holistic therapy, taking into account the mind, the body and the spirit of the person. Essential oils can connect with our body and help it to find the best way to solve its needs. The relaxing effects (parasympathetic activity) can be related to YIN and the activation effects (sympathetic activity) can be related to YANG.

RESUMEN

El objetivo general de esta tesis es analizar la eficacia de la aromaterapia (AR) para reducir las emociones negativas e incrementar el nivel de atención y rendimiento en el situaciones laborales y académicas. Se probará esta eficacia con cuestionarios y evaluando el equilibrio del Sistema Nervioso Autónomo a partir del análisis HRV. Para llevar a cabo este objetivo general, se realizarán dos estudios principales. Analizaremos los efectos de la AR sobre la variabilidad de la frecuencia cardíaca (HRV) como un marcador del equilibrio simpático/parasimpático desde el SNA.

El objetivo para el Estudio 1 fue analizar la eficacia de la AR parea reducir la ansiedad y mejorar la concentración en el estudio en estudiantes universitarios. La muestra fue de 12 estudiantes (edad entre 18 y 33 años). Analizamos los efectos del aceite esencial de albahaca *(Basil)* a corto término (1 hora) y a largo término (1 mes). Encontramos que la inhalación del aceite esencial de albahaca reduce las puntuaciones de ansiedad y mejora la variabilidad cardíaca (parámetros SDRR y RMSSD). La AR en una sesión corta o con una botella aromática colgada del cuello cada día durante un mes produce cambios emocionales y de HRV en los participantes. El objetivo del Estudio 2 fue analizar la eficacia de la AR para mejorar el rendimiento laboral y reducir el estrés en el lugar de trabajo. La muestra estaba formada por 42 trabajadores universitarios administrativos (edad: 42.21 años, SD=7.12; género: 10 hombres y 32 mujeres). Todas las sesiones fueron realizadas en un aula universitaria de ordenadores personales. Los participantes fueron asignados al azar a un grupo de aromaterapia (AG) y a un grupo control (CG), y fueron invitados a participar en una sesión específica solo una vez. Se sentaron frente a un ordenador. Durante el período de intervención, se pusieron en marcha algunos difusores de aceite y se mantuvieron funcionando a lo largo de la sesión con aceite esencial de *petit grain* para las sesiones del grupo AG y un aceite neutro (almendra) para las sesiones de CG. Al mismo tiempo, los participantes completaron una tarea de ordenador en un sitio web específico escribiendo con su teclado hasta que la terminaron. Los tiempos fueron diferentes para todos los participantes y se registraron en el sitio web como "Tiempo de ejecución". El grupo de aromaterapia realizó la tarea del sitio web

con el teclado 2, 28 min más rápido que la de CG ($p = 0,05$) y mostró una mejor variabilidad cardíaca (parámetros HF, SDNN y RMSSD). La inhalación de aceite esencial *Petitgrain* reduce el estrés y mejora el rendimiento en el lugar de trabajo. La AR mejora el tiempo de rendimiento al escribir con el teclado en una sesión simulada de trabajo de ordenador y produce algunos cambios mejorando la HRV.

Conclusiones generales: Esta tesis doctoral confirma que la aromaterapia (inhalar aceites esenciales) puede mejorar el rendimiento en el lugar de trabajo, puede ayudar a manejar emociones negativas y puede incrementar el nivel de atención en situaciones académicas y laborales. Nuestros resultados confirman que la inhalación de aceites esenciales produce este efecto equilibrando el sistema simpático / parasimpático del Sistema Nervioso Autonómo. El análisis de HRV es un buen marcador de este efecto. Este equilibrio del SNA puede explicarse por una acción combinada de los componentes principales de los aceites esenciales Petitgrain y Basil. Algunos de ellos tienen efectos sedantes, que pueden inducir cambios en la actividad parasimpática, y otros componentes tienen efectos de activación, que estimulan la actividad simpática. El efecto final puede ser una mejora de la condición mental y emocional por una combinación de reducir el nivel de estrés y aumentar el nivel de arousal de los participantes en términos de atención y alerta. También podemos explicar nuestros resultados según la Medicina Tradicional China (TCM). Los aceites esenciales pueden trabajar a través de una terapia verdaderamente holística, teniendo en cuenta la mente, el cuerpo y el espíritu de la persona. Los aceites esenciales pueden conectarse con nuestro cuerpo y ayudarlo a encontrar la mejor manera de resolver sus necesidades. Los efectos relajantes (actividad parasimpática) pueden estar relacionados con el YIN y los efectos de activación (actividad simpática) pueden estar relacionados con el YANG.

1. Introduction

1.1 The basic theory of Traditional Chinese Medicine (TCM).

1.1.1 Background of Traditional Chinese Medicine.

Traditional Chinese medicine (TCM) has a history of thousands of years (Hu ,Liu,2012). During its long course of development, it has absorbed much essence from other academic and cultural fields, including traditional Chinese culture, natural sciences, humanities and social studies, making it into a unique system of medicine with extraordinary theory, rich experience and significant therapeutic effect throughout history (Li, Xu 2011). TCM has contributed a great deal toward the development and prosperity of the Chinese nation. Now it has been disseminated to the other parts of the world and has been extensively studied.

TCM was deeply influenced by ancient Chinese philosophy (Hu, Liu,2012). It is formed by summarizing the precious experience of understanding life, maintaining health, and fighting diseases accumulated in daily life, production and medical practice. It does not only have systematic theories, but it also has abundant preventive and therapeutic methods to confront diseases. It also created a visceral manifestation theory and a meridian and collateral theory to explain the structure and physiological function of the human body and the developing law of disease. (Hu, Liu,2010; Lu, Jia, Cheng,2004)

1.1.2 Traditional Chinese Medicine Theory of holism.

TCM can be characterized as holism. The theory of TCM holds that the universe is a material integrity and is the result of the mutual opposition and unity between YIN

and YANG (Dong, Zhang,2001) The human body is an organic whole in which the constituent parts are structurally inseparable, functionally coordinated and interactive, and mutually influencing. The theory of TCM emphasizes on the integrity of the human body and the harmony between humans and their social and natural environment, but it also gives emphasis to the balance between the different systems and organs inside the body. TCM focuses on health maintenance and the treatment of diseases, specially remarking the importance of enhancing the body's resistance to diseases. (Zhou,2009; Dong , Zhang ,2001)

In TCM, illness is regarded as a state of disharmony between the individual and its natural and social environments. Accordingly, the aim of TCM is to help people regain a state of harmony. The curative and caring approaches focus not only on treating illnesses but also on counteracting the imbalances, which are the source of illnesses (Hu,Liu,2010; Dong , Zhang ,2001).

1.1.3 Theory of Yin and Yang.

The theory of yin–yang is one of the ancient Chinese philosophies that define the reciprocity of matters in the universe. The ancient Chinese thinkers found that every phenomenon has two opposing sides; they adopted yin-yang to explain any two matters in the universe that are opposed. Yin and yang are mutually opposed, but they are also connected. They contend against each other, restrain each other, and mutually repel each other. On the other hand, they are inter-dependent, they are the root of each other, and mutually generate and promote themselves. Moreover, yin and yang are not static; they are constantly growing or diminishing, or they even transform into their opposing side under certain conditions. Generally, things that are

mobile, exterior, rising, warm and bright pertain to yang, while things that appear static, interior, descending, cold and dark pertain to yin. Some examples are male and female, good and bad, top and bottom, right and left, and black and white, but the two contrary elements can be mutually transformed (Hu , Liu,2010; Dong ,Zhang ,2001)., (See figure 1)

Figure 1. Chinese Yin and Yang symbol, any two matters in the universe are opposed. (Zhou ,2008)

The philosophical theory of Yin and Yang permeates the theoretic system and clinical practice of TCM, which are used to explain the mechanism of balancing the functions of the human body. The theory of TCM holds that maintaining yin and yang in balance is the best way of preserving life. The imbalance between yin and yang is a source for all diseases. For improving health, TCM applies multiple natural therapeutic methods.

1.1.4 TCM multiple natural therapeutic methods.

TCM therapies are based on the traditional theory of Chinese medicine, through different ways of acting on the human body organs and blood or stimulate the human body acupuncture point, in order to clear the meridians, balance yin and yang, Qi and blood to adjust the role of organs. Chinese medicine practitioners can use different

treatments to achieve the purpose of treating diseases and health care of the patients.

1.1.4.1 Acupuncture.

Acupuncture is a kind of treatment that applies needles, heat, pressure, and other treatments to certain places on the skin, called acupuncture points, to cause a change in the physical functions of the body (Deng,2001). The use of acupuncture is part of TCM. It is a form of alternative medicine involving thin needles being inserted into the body. It is a key component of traditional Chinese medicine (TCM) and it is a very ancient system, having originated in China over 4000 years ago. It is deeply rooted in the Taoist philosophy which recognises two opposite and complementary energies, Yin and Yang, flowing through the whole of the nature of human beings. Yin and Yang energies are seen as a part of this whole, and flow through the body in a network of pathways called meridians, Yang energy passing down the back of the body and Yin energy up the front of the body. Both are maintained in a subtle and constantly changing balance. While this balance exists, and the energy flows freely, the body is healthy, but if a meridian is blocked at any point, there will be an excess or deficiency of energy, resulting in an illness. By inserting very fine needles at appropriate points on the meridians, the acupuncturist can remove the blockage and restore health (Deng,2001; Davis, 1992). (see figuer 2)

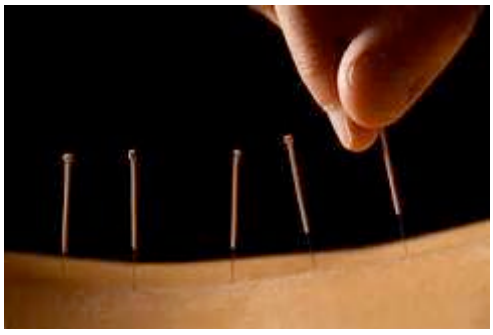


Figure 2. Needles being inserted into a person's skin

(http://www.southerncaliforniaacupuncture.com)(2016.9)

1.1.4.2 Tui Na (Acupuncture point and meridians massage)

The theory of meridians is an essential part of TCM. Meridians refer to the main pathways and collaterals refer to the network of branches. Meridians are longitudinal lines and collaterals are the transverse branches which run all through the body and connect all parts of the body together as shown in the figures below. Meridians are divided in two groups: twelve regular meridians and eight extraordinary meridians. The twelve regular meridians are connected with the six Zang organs (including pericardium) and the six Fu organs (see picture 3). The meridians are directly connected with the viscera and each Yin meridian matches a Yang meridian. The twelve regular meridians pertain to either Yin or Yang. One Yin meridian and one Yang meridian are internally and externally related to each other. For instance, the lung and the large intestine are internally and externally related to each other, so constipation can be treated by drugs that increase lung Qi. Thus, an illness can be treated by inserting needles in the acupuncture points located on the right meridians (Zhou 2008).

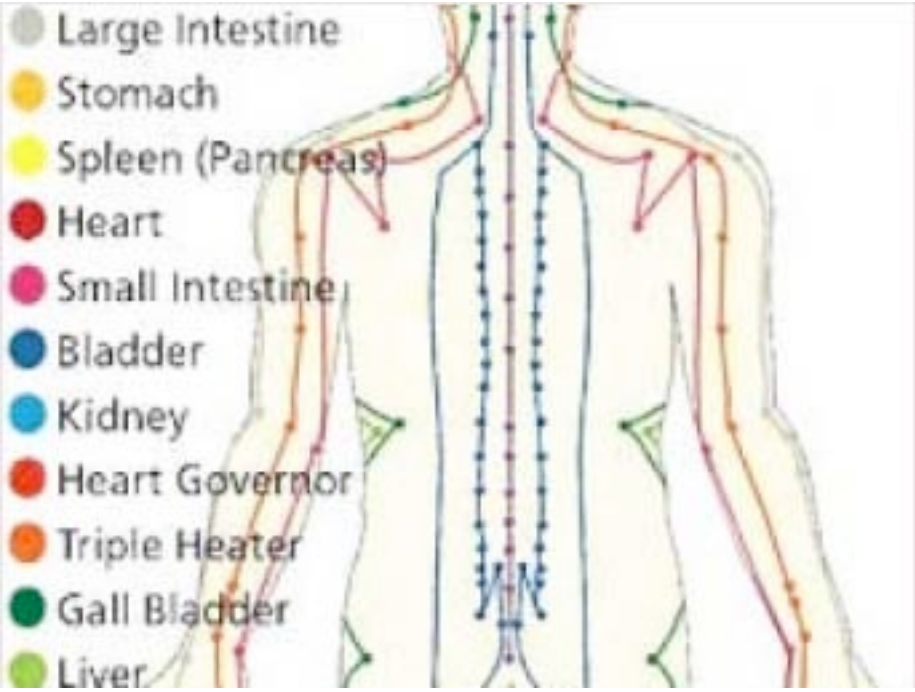


Figure 3: Twelve regular meridians related with the six Zang organs and the six Fu organs (https://healdove.com/alternative-medicine)(2016.9)

1.1.4.3 Negative emotion treatment of TCM

In TCM there are seven negative emotions that can cause disease. These seven emotions are normal feelings of human beings, but excessive changes of these emotions may damage the body and cause diseases. The seven negative emotions are joy, anger, anxiety, contemplation, sorrow, terror and fear. Every emotion is controlled by different organs. For example: the heart stores joy. Laughing indicates happiness, but excessive laughing may cause diseases. The heart stores the spirit, excessive laughing disperses the spirit and that is why it is said that excessive joy is harmful to the heart. Excessive anger is related with liver qi. The liver in related with the emotions of joy and tenderness; excessive anger will damage liver qi and result in people losing temper. Excessive anxiety and grief are related with lung and spleen qi. An excess of anxiety and grief blocks qi flow, eventually damaging the lung which governs qi and

also damages the spleen due to indigestion. Terror and fear damage kidney qi. Terror and fear indicate extreme nervousness and timidity, usually caused by deficiency of kidney qi or blood. Nevertheless, emotions could be influenced by each other: anger damages the liver and sorrow surpasses anger; joy damages the heart and terror surpasses joy; thinking damages the spleen and anger surpasses thinking; anxiety damages the lung and joy surpasses anxiety; terror damages the kidney and thinking surpasses terror (Zhou 2008). Thus, this is a Chinese pattern to treat emotional disorder.

1.1.4.4 CHM（Chinese herbal medicine）

Chinese herbal medicine, which is the most important branch of TCM, is widely used in China and it is increasingly being used worldwide. CHM is empirically based and has a history of over 4000 years. The biological ingredients of herbal remedies are extracted from natural substances: plants, animal parts, shells, insects and even stones and minerals. In Chinese herbalism, every herb has its own properties. Chinese herbalists believe that illnesses can be effectively treated by combining herbs, taking into account their various characteristics and the patient's overall status. The composition of the formula and the dosage of the individual ingredients depend on the signs and symptoms of the patient, which is the basic principle of CHM. These formulas can be modified to fit specific individuals more closely (Ruan , LAI , ZHOU 2006).

With the development of society and the constant innovation of science and technology, it has been discovered that the active ingredients of plants can be purified by distillation technology, which opens up a new direction for ancient Chinese medicine therapy. Aromatherapy, which uses a large number of aroma herbs as a basis

for any treatment, will become a new area of TCM.

1.2 Aromatherapy.

1.2.1 Background of aromatherapy.

Aromatherapy (AT) is an ancient herbal medicine tradition and it was used in some ancient countries, such as Egypt, China and India, thousands of years ago. AT is a kind of natural treatment method utilizing the chemical structure and effects of plants essential oils, which extracted from herbs, roots, leaves, flowers, and other plant parts, they can be used to treat various diseases (Cooke and Ernst, 2000).

AT has significant therapeutic potential because each essential oil has a unique combination of chemical compounds that interact with the body's chemistry and thereby affect specific organs, systems and the body as a whole. For example, some studies have shown that certain essential oils decrease anxiety-related behaviour and improve some illnesses in humans and animals (Koulivand, Khaleghi, Gorji,2013).

AT has grown in importance as an area in alternative medicine with proven high efficacy in reducing stress and improving mood disorders (Souto-Maior, Carvalho, Morais *et al*, 2011) Recently, AT is used by more and more people around the globe, and clinical research into this field is also increasing every year. Some studies reported that inhaled pepper oil induced an increase in adrenaline concentration, while inhaled rose oil decreased the adrenaline level (Haze, Sakai , Gozu ,2002). Some studies have also shown that stragon oil, or grapefruit oil, stimulates effects on the sympathetic and parasympathetic activity. Ocimum basilicum L has been traditionally employed as a medicinal herb in the treatment of headaches, coughs, diarrhoea, constipation, warts, and/or kidney malfunction (Rajesh, Joshi, 2014)

1.2.2 Effect of Aromatherapy

Aromatherapy has beneficial effects on our body in two main ways:

1.2.1.1 The effect of the Physical symptoms

Some studies show that aromatherapy is useful to reduce physical symptoms, such as pain, cancer, chronic diseases, antibacterial and antiviral diseases. Małachowska B, Fendler W discovered that oils reduce autonomous response to pain sensation during self-monitoring of blood glucose among children with diabetes(Małachowska , Fendler , Pomykała, *et al* ,2016. Shin ES found that massage with aroma can relief the symptoms in people with cancer (Shin, Seo *et al*,2016); Marchese A, Orhan IE found that antibacterial and antifungal activities of thymol (Marchese, Orhan *et al*, 2016). In Vitro Antibacterial Activity of Essential Oils against Streptococcus pyogenes (Sfeir, Lefrançois, *et al* ,2013)

1.2.1.2 The effect of emotions on health

Essential oil can be used to change the mood, particularly those which are antidepressant and cheering. Virtually any of the uses of essential oils in massage, baths, perfumes and other methods have a beneficial effect on symptoms such as stress, fear, anxiety, withdrawal and depression (Price, Price 1995).

Franco Land and his colleagues found that lavender fleur oil and unscented oil can reduce preoperative anxiety in breast surgery patients (Blank, Dugan ,2016); Hosseini and his colleagues found that inhaling lavender essence could reduce the level of anxiety and blood cortisol in candidates for open-heart surgery (Hosseini, Heydari 2016). Chamine and Oken BS found that aroma have effects on physiological and

cognitive function following acute stress (Chamine and Oken 2016).

1.2.3 Essential oils: The main component of Aromatherapy

Essential oils are the principal component of aromatherapy. They are concentrated volatile compounds formed as secondary metabolites in aromatic plants and characterized by a strong odour (Solgi, Ghorbanpour,2014). Their biological effects may be the result of the synergistic effect of all the molecules together or only of the main molecules found in larger concentrations of these oils. The effectiveness of essential oils is believed to be derived from each of the essential oils with unique chemical components (Bakkali,averbeek,,Averbeek,2008). Different essential oils depending on the chemical components affect our emotions. For example, on the one hand, erpenes and sesquiterpenes, which are the biggest chemical family in essential oil, could stimulate sympathetic activity. Pinene, myrcene, limonene, caryophyllene and other components were detected in pepper oil, estragon oil, fennel oil and grapefruit oil (Baik,Kim , Hyun *et al ,* 2008)

On the other hand, ethers and alcohols induce inhibition of sympathetic activity. Methyl chavicol, anethole, citronellol, geraniol, nerol alcohol, patchouli alcohol and other components were detected in rose oil and patchouli oil (Haze, Sakai, Gozu,2002). Our study compared two groups of people working on a website and found that the use of *Petitgrain* can make work performance 2.28 min faster. All participants in all groups showed a decrease from PRE to POST in the emotion scales (Huang, Capdevila,2016).

Pierre Franchomme, a France chemist, invented the structure-effect diagram of essential oils which was a decisive contribution to modern aromatherapy. The

structure-effect diagram was created to show the impact that the tendency to attract or to donate electrons has on the properties of an essential oil component.

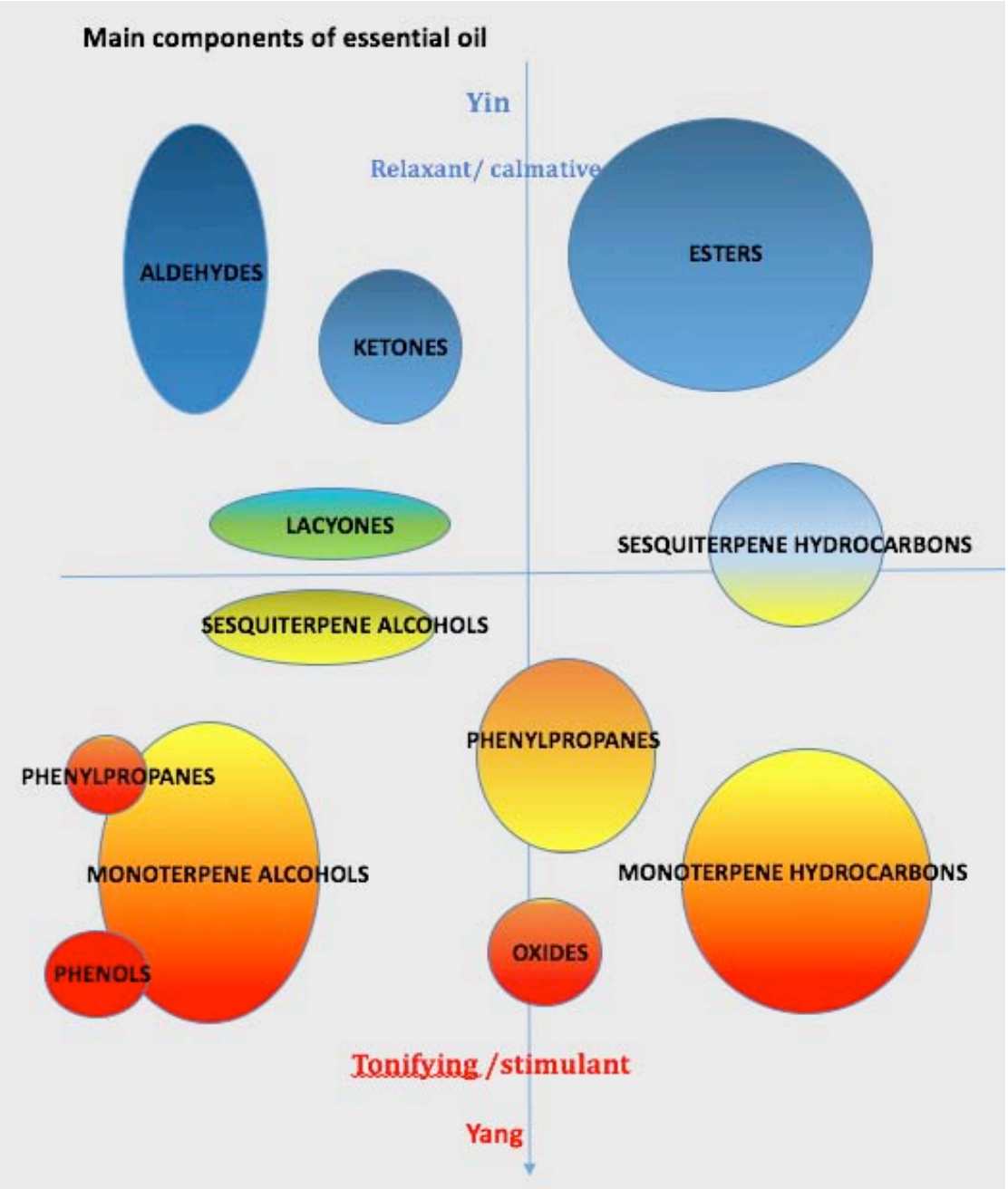


Figure 4: The structure-effect diagram of the main components of essential oil (The main components of essential oil can be placed in a coordinate system according to their chemical qualities and their lipophilic or hydropgilic nature)

The structure-effect diagram allows us to connect a physically measureable parameter with the effect of the oils. It provides a tangible model for approaching the

scientific concepts of the complicated essential oils chemical composition (Franchomme, Jollois, penoel, 2001). (See Figure 4)

1.2.4 Pathways of aromatherapy working in the body.

The essential oil can be absorbed into the body through four main channels (see Figure 5): a) through the skin, b) through anus and vagina suppositories, c) through the oral system d) through the olfactory system (Price, Price ,1995). A) skin massage: Archaeological evidence of massage has been found in many ancient civilizations including China, India, Japan, Korea, Egypt, Rome, Greece, and Mesopotamia. It involves working and acting on the body with pressure, which can be structured, unstructured, stationary, or moving and with tension, motion, or vibration, done manually or with mechanical aids (Davis 1992). Massage can be applied with the hands, fingers, elbows, knees, forearms, feet, or a massage device. Depending on the application and technique used, massage is used to promote relaxation and well-being (Price, Price 1995; Davis 1992) and it is beneficial in treating sports injuries (Franchomme, Jollois, penoel,2001) and other problems affecting the musculature of the body such as postural misalignment and many painful conditions. Massage following the meridian and acupuncture point or with essential oil is an efficient way to improve our body's health. (Price, Price 1995; Davis 1992) B) Suppositories and pessaries are useful in cases of irritable bowel syndrome, haemorrhoids, vaginal infections and candida. This way essential oils have direct access to the bloodstream with little effect on metabolization. Since essential oil is directly injected into the bloodstream it is important to be very careful with the dose, which should not exceed 6 drops. Toxic or irritant essential oils should not be used. C) Ingestion is the main

route employed by primatologists and doctors in France. It is useful in cases of antibacterial and antiviral, but it is not common in other countries, since it is normally used professionally as a medicine. When ingested orally, the greater part of the essential oil is absorbed by the mouth or the throat, the oesophagus, the stomach or the duodenum and the small intestine as, it travels along the digestive path. From there, essential the oil travels to the liver, where it is metabolized (Price , Price 1995; Kurt Schnaubelt 1998).

When oils are ingested through the olfactory system, the mechanism of action of inhaled aroma starts with the absorption of volatile molecules through the nasal mucosa (Susanna, Alina and Dalila , 2104). Then the odour molecules of essential oils are transported to the brain by the olfactory sensory neurons in the nasal cavity. Odour molecules are then transformed into chemical signals, which move towards the olfactory bulb, and possibly to other parts of the limbic system. They stimulate smell memory work, influence memory, thoughts and emotions (Krusemark,Novak, Gitelman,*el ct*,2013) by interacting with the neuropsychological framework to produce psychological effects. Even a small amount of molecules inhaled by respiration causes an indirect physical effect by activating the memory or by infiltrating the blood. (Krusemark ,Novak, Gitelman,*el ct*,2013; Robins ,1999) (see Figure 5)

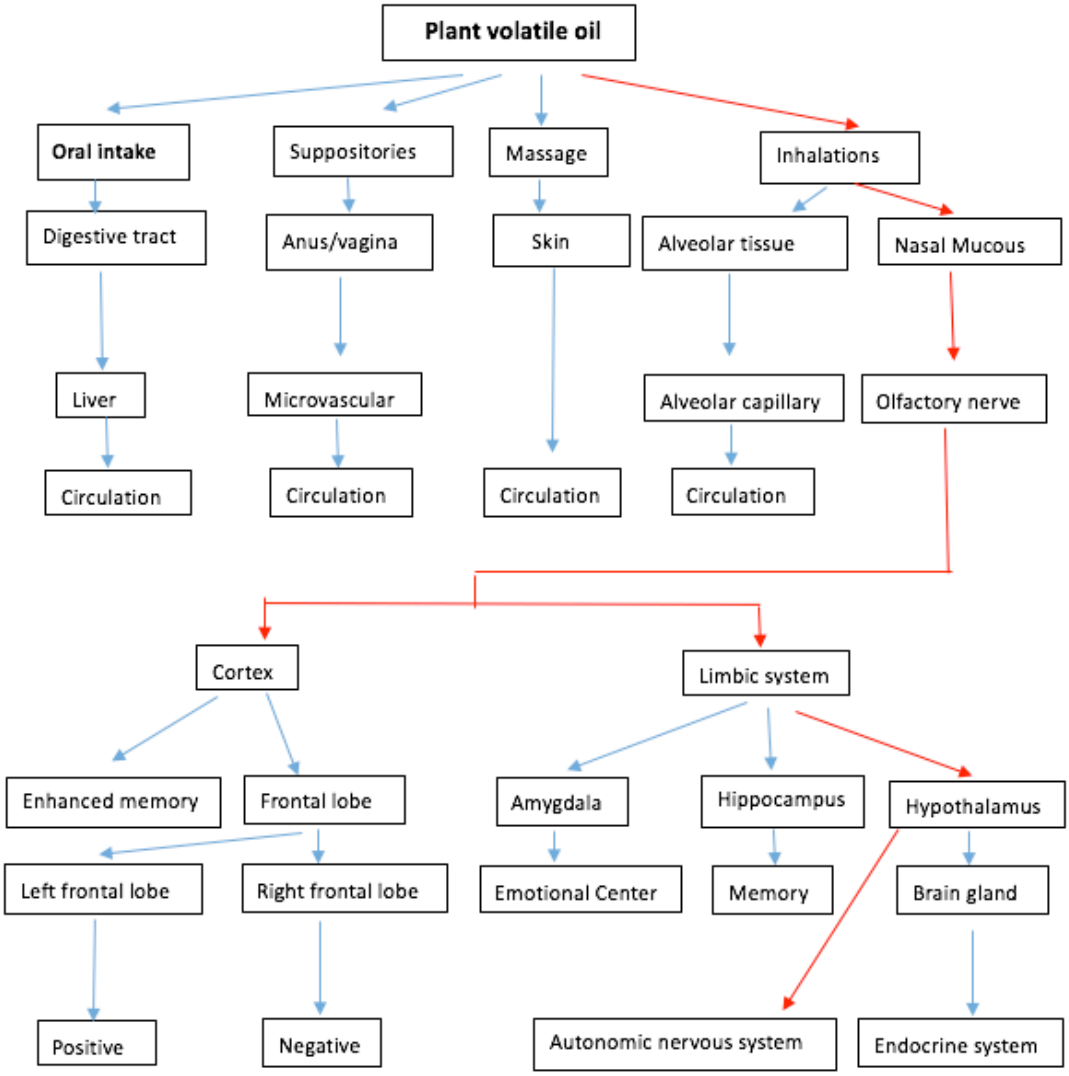


Figure 5: Four main pathways though which essentials oils can be introduced into the body.

1.3 Two main systems of aroma inhaling.

1.3.1 The olfactory system.

The olfactory sense is phylogenetically the oldest sense. It has a unique intimacy with emotions, unlike other senses. Olfactory neuroanatomy is intertwined, via extensive reciprocal axonal connections, with primary emotion areas including the amygdala, hippocampus, and orbitofrontal cortex (OFC). Olfactory stimulation can directly activate amygdala neurons, bypassing the primary olfactory cortex, before arriving at the secondary (association) olfactory cortex situated in the middle of the

OFC (Kruemark ,Novak,Gitelman *el ct*,2013). (See Figures 6)

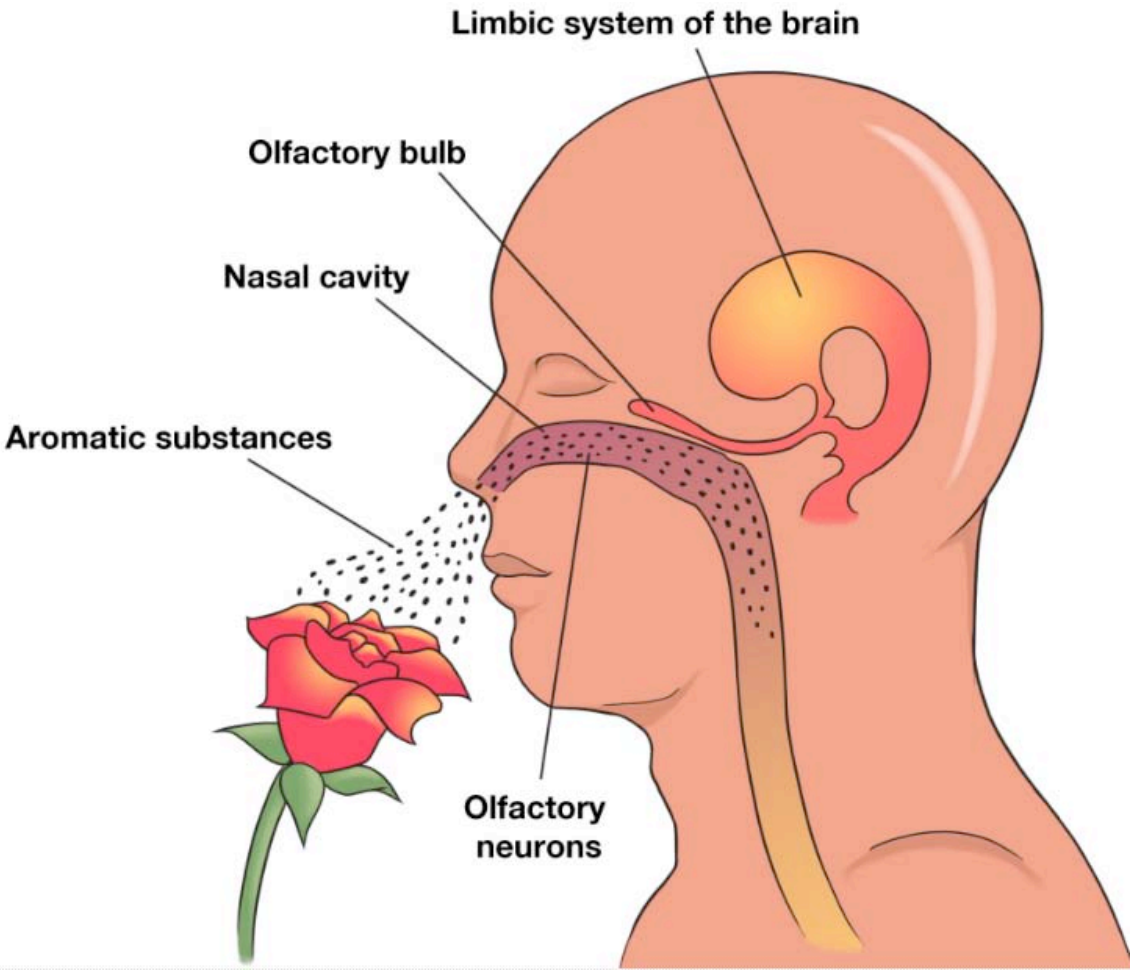


Figure 6: Diagram of the Olfactory System. The patch of neurons located at the top of the nose feed into the olfactory bulb which sends signals directly to the brain.
http://www.gridgit.com/postpic/2010/11/how-the-olfactory-system-works_502244.jpg(2011)

The sense of smell plays an important role in the physiological function of the individual because its response is immediate and it is directly extended to the brain; it seems to be related to emotional behaviour. The odour molecules of essential oils are transported to the brain by olfactory sensory neurons in the nasal cavity; thus, they stimulate smell–memory work and influence memory, thoughts and emotions (Lledo,Gheusi,Vincent ,2005).

1.3.2 The Autonomic Nervous System (ANS).

The odour molecules of essential oils are transported to the brain by olfactory

sensory neurons located in the nasal cavity and stimulate the autonomic nervous system. Thus, another of the beneficial effects of aromatherapy is the ANS balance, which manages the sympathetic- parasympathetic interaction (Janig,1989).

The autonomic nervous system (ANS) is a division of the peripheral nervous system that influences the function of internal organs. The autonomic nervous system controls unconscious actions and regulates bodily functions such as heart rate, digestion, respiratory rate, pupillary response, urination, and sexual arousal (Schmidt ,Thews ,1989). The autonomic nervous system is composed of two parts, the sympathetic nervous system and parasympathetic nervous system, which function primarily in opposition to each other. The sympathetic nervous system starts in the spinal cord and travels to a variety of areas of the body. Its function appears to be preparing the body for the kinds of vigorous activities associated with “fight or flight,” that is, with running from danger or with preparing for violence, associated with energy mobilization. The parasympathetic nervous system has its roots in the brainstem and in the spinal cord of the lower back. Its function is to bring the body back from the emergency status which the sympathetic nervous system puts into. It is also associated with vegetative and restorative functions (McCorry,2007). Some studies show that the nervous system has a particularly powerful role to play in our emotional life (C. George boeree,2002; Shin ,Liberzon 2010).

1.4 Heart Rate Variability (HRV)

HRV refers to the variation in the time interval between consecutive heartbeats (the RR interval). HRV analysis is a valid and reliable method to check the ANS balance and it can be used to evaluate the AR effectivity (Chang, Shen,2011; Cervantes, Rodas,

Capdevila ,2009)

Finally, it should also be taken into account that there is a trend to use uncommon EOs, often derived from wild plants which have a tendency to produce numerous cultivars with different chemical compositions. The different chemotypes have often not been tested toxicologically, and further problems could possibly derive from this in an uncontrolled market.

2. APPROACH, OBJECTIVES AND HYPOTHESIS

2.1 Approach

In the Introduction part of this thesis we found that numerous studies have shown that negative emotions are associated with bad health, such as cardiovascular disease, diabetes, cancer, musculoskeletal problems, or alcohol dependence, and even with mortality. In fact, negative emotions constitute the most potent risk factor for suicide. Depression, stress and anxiety are the most prevalent negative emotions related with health problems in the world that result in an enormous burden on the health care system. The World Health Organization (WHO) has envisaged that negative emotions related with chronic psychiatric disorders will become the second leading cause of premature death or disability worldwide by the year 2020 (Reddy ,2010).

In the European Union, 350 million work days are lost because of negative emotions related problems, resulting in an overall cost of at least 20 billion euros every year. This problem has been noted by the European Commission (Taelman,Vandeput , Vlemincx , *et al* 2011).

Increasingly, experts call for the implementation of human health promotion programmes. In particular, people who suffer from negative emotions problems should be identified at an early stage so that preventive action can be taken. Thus, many depression, stress and anxiety reduction related methods have been investigated and various approaches have been involved in negative emotions management. Aromatherapy is one of these approaches thanks to its easy implementation and effectiveness (Chang, Shen 2011).

At present, aromatherapy is used by more and more people around the globe,

and clinical research into this field is also increasing every year. This discipline has grown in importance as an area in alternative medicine with proven high efficacy in improving negative emotions and mood disorders. But there are some important questions to answer about the action mechanisms of Aromatherapy and about the scientific evidence of its effects.

2.2 Objectives

The general aim of this thesis is to analyse the aromatherapy (AR) efficacy to reduce negative emotions and increase the attention level and performance in the workplace and in academic situations. Its efficacy will be tested through questionnaires and by checking the balance of the Autonomic Nervous System through HRV analysis.

To carry out this general objective, we conducted two main studies (Study 1 and Study 2) with their respective pilot-studies (Pilot-Study 1 and Pilot-Study 2). The specific objectives for each study are:

2.2.1 Objectives for Study 1.

1. To analyse the AR efficacy for reducing anxiety and improving study concentration in university students.

2. To analyse the long-term effect of different AR oils on negative emotions and attention level in academic situations.

3. To explain the AR acute effects during 1-hour inhalation.

2.2.2 Objectives for Study 2.

4. To analyse the AR efficacy to reduce workplace stress and improve work

performance.

5. To analyse the effect of different AR oils on stress and performance in the workplace.

Objective for both studies:

6. To analyse the AR effect on HRV, used as a marker for the sympathetic /parasympathetic balance.

2.3 Hypothesis.

1. Inhalation of *Ylang ylang* or *Sweet Basil* essential oils can reduce academic anxiety and improve study concentration in university students (Study 1).

2. Inhalation of *Elemi* or *Petitgrain* essential oils can reduce stress and improve performance in the workplace (Study 2).

3. Inhalation of essential oils has its deepest acute effects at 20-25 min after starting inhaling (Study 1).

4. AR has short-term efficacy (1 hour) and long-term efficacy (1 month) to reduce negative emotions (both studies).

5. Inhalation of essential oils can reduce negative emotions by balancing the sympathetic/parasympathetic system of the Autonomic Nervous System, and HRV analysis can be a marker of this effect.

6. Inhalation of *Ylang ylang, Sweet basil, Elemi* or *Petitgrain* will increase the HRV and will reduce the questionnaire scores of negative emotions.

3. General Material and Methods.

3.1 Questionnaires.

We will resume the cognitive instruments we used in all studies:

1) A global “Feeling Scale” askiing for “How do you feel today”. Participants had to choose one value from 0 (“very bad”) to 10 (“very good”) scales. The Feeling Scale will be used to assess global the participants feeling state before and after sessions.

2) A short version of Profile of Mood States (POMS) (Shacham ,1983). The POMS consists of 15 items, three for each subscale (*tension, depression, hostility, vigour and fatigue*). The *tension* scale indicates that the higher the score, the tenser the participant is; the *depression* scale indicates that the higher the score the more sadness the participant feels; the *hostility* scale indicates that the higher the score, the more hostility towards others the subject feels; the *vigour* scale is a positive scale and suggests that the higher the score, the more energy the participant has; the *fatigue* scale indicates that the higher the score, the more fatigue the participant feels. All these five scales contain three items that are scored according to a scale from 0 (“nothing”) to 10 (“very much”). The POMS will be used to assess mood states before and after the sessions.

3) The State-Trait Anxiety Inventory (STAI) (Spielberger, Gorsuch, Lushene , 1970). This instrument is divided into two scales, each having 20 questions. The first subscale measures state-anxiety (STAI-S) and the second measures trait-anxiety (SATAI-T). The participants were asked to rate the intensity of each symptom on a scale from 0 (“not at all”) to 3 (“very much”), resulting in scores ranging from 0 to 60. The scale indicates that the higher the score, the more anxiety state or trait the

participant has. STAI-T scale will be administered immediately prior to the first session and immediately after the last session. STAI-S scale will be used to assess anxiety state before and after the sessions.

4) SF-12v2 (SF12) Health Survey is a 12-item short form of SF-36 global instrument (Schmidt,Thews, 1989) The 12 answers are based on a Likert scale from 2 to 6 answer options, which permit to obtain two global scores: Physical Component (PCS_SF12) and Mental Component (MCS_SF12). SF12v2 will be administered immediately prior to the first intervention and immediately after the last intervention.

3.2 Heart Rate Variability (HRV) analysis: instruments and procedures.

We performed HRV analysis in all studies and sessions. Participant's session ratings of continuous heart rate (RR or beat to beat cardiac intervals) were collected and analysed with FitLab® Team system (HealthSportLab.com, Barcelona, Spain). It consists of an application that runs in a mobile device (iPad, Apple) connected via Bluetooth (BTv4) with several cardiac chest bands (H7 model) to obtain RR intervals, and connected via wireless with a remote server (HealthSportLab.com). The system permits to perform synchronized recordings of all participants in each session and check at real time the quality of the data. Typically, participants are asked to remain at rest in the same position, without speaking or making any movements, and heart rate variability data is registered continuously following a natural breathing. FitLab® System uses Polar technology which has been validated for detecting RR intervals at rest (Parrado, Garcia, Ramos, Cervantes, Rodas & Capdevila, 2010). The digitized signal is filtered, in accordance with common standards, and processed by the FitLab® System software to obtain and filter the RR series, and calculate several HRV parameters. For

the time domain analysis, these HRV parameters are: the mean of RR intervals (RRmean); the standard deviation of all RR intervals (SDRR); and the root mean square of differences (RMSSD) of successive RR intervals. For frequency domain analysis, all RR series were re-sampled at 3 Hz using a cubic spline prior to the HRV analysis. The power spectrum of the re-sampled time series was estimated using the Fast Fourier Transform after removing the mean of the time series and multiplying the time series by a Hann window. The HRV frequency parameters are: the power of the very low frequency band (VLF), which was estimated by integrating the power spectrum for frequencies lower than 0.04 Hz; the power of the low frequency band (LF), computed in the band 0.04 – 0.15 Hz; and the power of the high frequency band (HF), computed in the band 0.15 – 0.4 Hz. Additional calculations included the LF/HF ratio, LF and HF values expressed in normalized units (LFnu and HFnu). The calculation of these indices is consistent with the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electro physiology (1996) (Moreno, Ramos, Castro and Capdevila 2015).

To control these variables, which could influence heart rate variability, in all sessions, participants were asked not to consume any food of beverages containing alcohol or caffeine after the evening of the day preceding the session. They were also asked not to do vigorous physical activity during 24 hours before being tested.

3.3 Aroma instruments and procedures.

3.3.1 Aroma instruments

- Essential Oils (100% pure):

1) Ocimum basilicum, L, (100% pure). Abbreviation = OBL; English name: *Sweet*

Basil.

2) Citrus aurantium bigarade. Abbreviation =CAB; English name: *Petitgrain*.

3) Canarium luzonicum. Abbreviation =CL; English name: *Elemi.*

4) Cananga odorata; Abbreviation =CO; English name: *Ylang ylang.*

All essential oils were diluted to 2% before to be used.

-Vegetable oil (100% pure):

Prunus amygdalus var. dulcis. Abbreviation =PAV; English name: *Sweet Almond*

Sweet Almond is a kind of vegetable oil with natural smell. It was diluted to 2% before being used.

In our text we will use the English oil name. In tables and figures we will use abbreviations of Latin names.

3.3.2 Aroma Intervention procedures.

Aroma intervention was conducted once a week, a total of 4-6 weeks in the lab, in the work site or at home, depending on the study. HRV parameters were recorded during lab sessions.

a) An Ultrasonic Ionizer Aroma Diffuser was used for aroma evaporation in the lab.

b) Aroma necklaces were used for aroma evaporation at home or in the work site.

Detailed experimental procedures were as follows.

(1) Basic aroma interventions were conducted on workdays in the morning. Smoking and drinking alcohol or coffee was forbidden during the six hours before the aromatherapy session.

(2) Lab sessions: Participants were asked to rest for 5 minutes before performing

any task. Then, a 45-90 min aroma inhalation took place.

(3) Aroma inhaled at home: participants conducted 3 hours of aroma inhalation in their home wearing the aroma necklace.

4. Pilot Study 1.

4.1 Objective

the aim is to conduct a pilot study for determining the procedures and characteristics of aroma methods, based on the meridian system, to reduce anxiety and to improve attention control in a university environment. This information will be useful to conduct the definitive study later.

4.2 Sample

The participants were 3 postgraduate university students (aged 24, 26 and 33; gender: 1 male and 2 females). All participants agreed to collaborate voluntarily and provided informed consent before participating in the pilot study. They met the following inclusion criteria: 1) no clinical diagnosis of autonomic dysfunction; 2) no clinical diagnosis of respiratory, cardiovascular, endocrine or systemic disorder; 3) no problems with their olfactory function; 4) None of the participants had been clinically diagnosed with diabetes mellitus, hypertension, hyperlipidaemia, asthma, cardiovascular disorders, or any other endocrine or systemic disorders that could affect the autonomic nervous system. Five participants completed two initial lab sessions, four completed the third session and three completed the last three sessions.

4.3 Instruments

For this pilot study we used the FitLab Team system (see part 3.2) to analyse HRV and the questionnaires STAI-S, POMS, the Feeling scale, SF12v2 and STAI (see part 3.1). We also used lifestyle questionnaire interviews as qualitative research instruments to collect data about the lifestyle behaviours of participants during those days that they

used the aroma.

Participants interviews took place during the lab sessions. Themes explored included: symptoms, effects, management of the aroma, willingness, sleeping habits and lifestyle behaviours (see items in Table 1). Interviews were digitally transcribed and analysed. This information helped to identify aromatherapy specific situations and challenges for a more effective management (Chien, Cheng, Liu,2012).

Table 1. interview of lifestyle questions to collect data about the lifestyle behaviour of participants during the days that they used aroma at home.

Code	Question	Result
1	How was your sleep and dream pattern?	Good/Average/Poor
2	Have you eaten regularly?	Yes/No
3	How was your stool ?	Good/Average/Poor
4	What time did you usually smell the oil ?	Morning/Afternoon/Night
5	How was your learing attention when smelling the oil?	Good/Average/Poor
6	How was your learning attention during this week?	Good/Average/Poor
7	Do you like the aroma?	Yes/No
8	Do you like this way to use oil?	Yes/No
9	Did you feel any change on your emotions after smelling the oil?	Yes/No
10	Are your emotions more positive or more negative after smelling the oil?	Positive/Negative

4.4 Procedure

4.4.1 General procedure for all sessions.

We did not inform participants about which fragrance or oil we were going to use for the study. We conducted this pilot study through 4 sessions in different weeks and conditions (See Table 2).

Participants went to the Lab in 4 group sessions distributed in different weeks. All sessions were performed in the morning between 9:00AM and 11:00AM in a quiet lab environment. In all the sessions the ambient temperature was between 22 and 25 ºC.

In general, the intervention in each session consisted in a 20-min self-massage with a specific oil on a specific acupuncture point and in a 60-min aroma inhalation of a specific oil. All participants performed a total of 4 sessions in 4 weeks.

Table 2. Characteristics of the 4 sessions conducted in the Pilot Study 1 with students.

(CO: *Ylang ylang*; OBL: *sweet Basil*).

Session	**N**	**Time**	**Place**	**Oil**	**Use way**
Session 1	3	60 min	Lab	CO	Smell and massage
Session 2	3	60 min	Lab	CO	Smell
Session 3	3	60 min	Lab	OBL	Smell and massage
Session 4	3	60 min	Lab	OBL	Smell

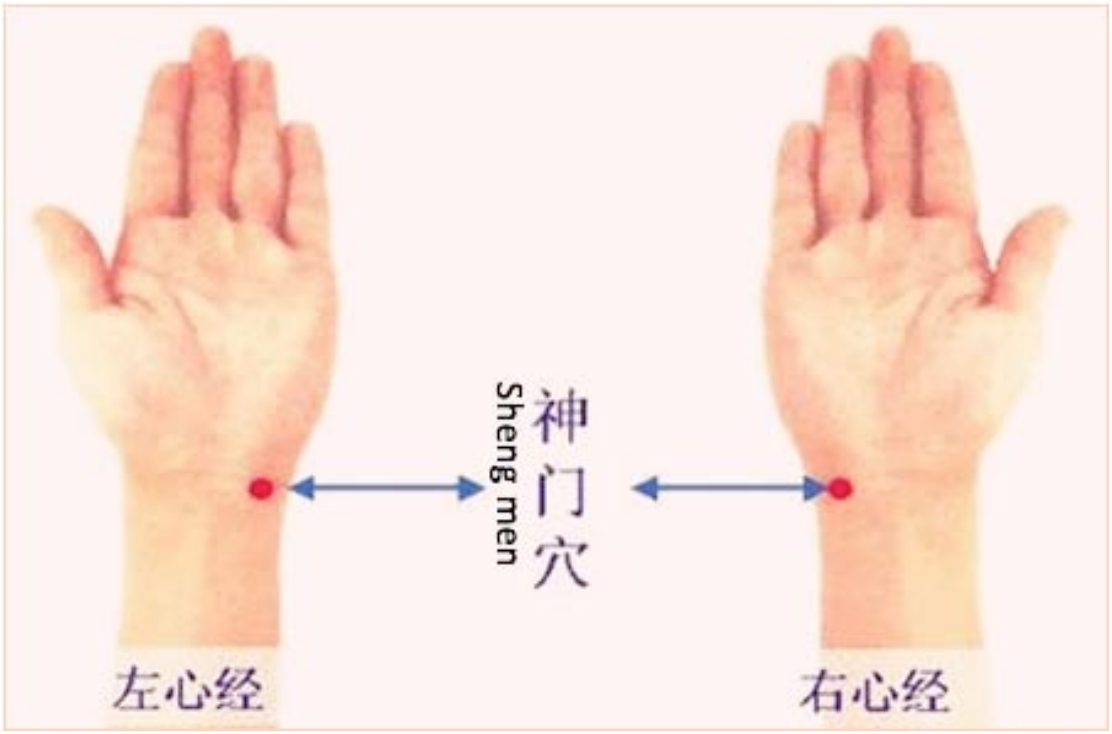
1) When the group of participants arrived to the lab they were asked to rest in a sitting position in front of a circular table, like in their classroom. In general, they followed the steps below (See the Figure 8):

2) Participants were required to fill out the PRE questionnaires. (STAI-S, POMS, SF12v2 and the Feeling scale).

Participants were asked to wear the cardiac chest band (connected via Bluetooth to an iPad with FitLab software) and sit at rest around the table.

3) HRV-Pre (before aroma) was recorded for 5 minutes with the participants at this rest situation: sitting, eyes opened, hands on their knees, doing no activity at all, breathing naturally and without speaking.

4) A specific aroma was scattered in the environment and participants could be required to self-massage an acupuncture point of their body in session 1 and 3 (see Figure 7). At the same time, they were inhaling the aroma and the HRV was recorded during a minimum of 40 min.



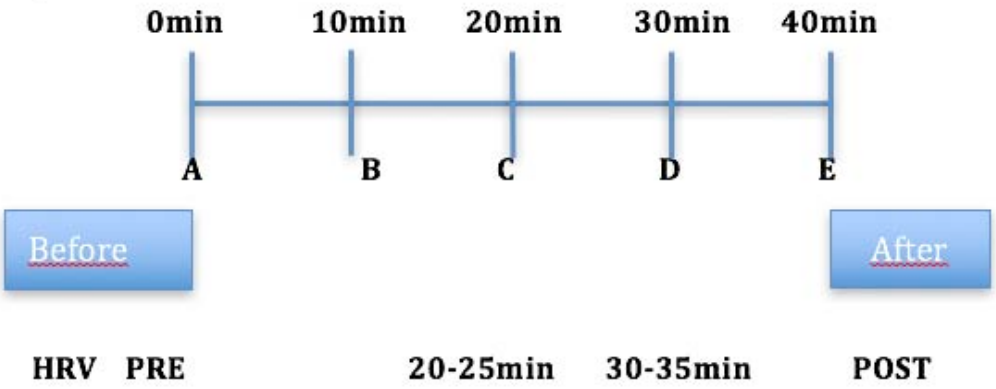
Figuer 7. TCM Acupuncture points of "*Shengmen*"

http://www.taozhy.com/ShuJuKu/XueWei/104.thtml (2012)

5) Participants were required to perform a 5-min HRV Postest (sitting, eyes open, hands on their knees, doing no activity, breathing naturally and without speaking).

6) Participants were asked to fill out the POST questionnaires (STAI-S, POMS and the Feeling scale.

7) A short interview was performed for participants who used the aroma at home. (Except for Session 1)



Figuer 8. General experimental procedure and data collection for Pilot Study 1.

STEPS: (1) Before: State–Trait Anxiety Inventory (STAI) and Profile of Mood States (POMS) questionnaires. Participants were asked to (2) wear the cardiac chest band. (3) PRE: 5 min-HRV-Pretest. (4) A) Starting the oil inhalation and/or hand massage; B) Massaging the other hand; C) (During 1) Finishing massage and starting the 5 min-HRV test; D) (during 2) 5 min HRV-Test; E) Finishing inhalation and (5) starting POST: 5 min-HRV Postest. (6) After: STAI and POMS. (7) Short interview.

PRE: 0min: 5min PRE

During 1: 20 to 25 min

During 2: period between 30 to 35 min

POS: 5 min POS

4.4.2 Specific procedure for Session 1.

Participants followed the general procedure and the steps 1 to 3 explained above. At the beginning of the fourth step**,** *Ylang ylang* aroma was scattered in the room through the diffusers (see part 1.3) for all the remaining session. This allowed a proper aroma inhalation throughout the complete lab room. In the fourth step, participants were also required to massage themselves on the left hand on the acupuncture point

of "*Shengmen*" 10 min (see Figure 7). This massage was performed clockwise with the right hand. Then, a clockwise massage with the left hand on the acupuncture point of "*Shengmen*" on the right hand was performed another 10 minutes. After this time the participants stopped the massage and sat at rest while completing the remaining session steps.

At the end of the session, participants were asked to wear a necklace (See Part of 3.3.2) for 7 days and for inhaling the *Ylang ylang* oil during 3 hours per day at home. The specific instructions were: "you have to inhale the aroma for 3 hours at home by opening the aroma necklace whenever you prefer and you have to write down how you feel during the inhalation"

4.4.3 Specific procedure for Session 2.

Participants followed the general procedure and steps 1 to 3 explained previously. At the beginning of the fourth step, *Ylang ylang* oil was scattered in the room through the diffusers (See part 3.3.2) for all the remaining session. This allowed a proper aroma inhalation throughout the complete lab room. Then, participants followed the general procedure and steps 5 to 7 explained previously.

During the week between session 1 and session 2, all participants used a necklace at home to inhale the *Ylang ylang* oil whenever they preferred, at least 3 hours a day for a period of 7 days in total.

4.4.4 Specific procedure for Session 3.

Participants followed the general procedure and steps 1 to 3 explained previously. At the beginning of the fourth step, *Sweet basil* oil was scattered in the room through the diffusers (See part 3.3.2) for all the remaining session. This allowed a proper aroma

inhalation throughout the complete lab room. In the fourth step, participants were also required to massage themselves on the acupuncture point of "*Shengmen*" on their left hand for a period of 10 min (see Figure 8). The massage was performed clockwise with their right hand. Then, a clockwise massage with their left hand on the acupuncture point of "*Shengmen*" of their right hand was performed another 10 minutes. After this time the participants stopped the massage and sat at rest while completing the reaming session steps.

During the week between session 2 and session 3, all participants used a necklace at home to inhale the *Sweet basil* oil whenever they preferred, at least 3 hours a day during a period of 7 days in total.

4.4.5 Specific procedure for Session 4:

Participants followed the general procedure and steps 1 to 3 explained above. At the beginning of the fourth step, *Sweet basil* oil was scattered in the room through the diffusers (see part 3.3.2) for all the remaining session. This allowed a proper aroma inhalation throughout the complete lab room. Then, Participants followed the general procedure and steps 5 to 7 explained above.

During the week between session 3 and 4, all participants used their necklace at home to inhale the *Sweet basil* oil whenever they preferred, at least 3 hours a day during a period of 7 days in total.

4.5 Results

In this part, we will present the most relevant results of this pilot study for obtaining the key information to design the definitive study (Study 1). Among the 6 initial participants, 3 participants completed all sessions of the pilot study. The initial

participants constituted a heterogenic sample: postgraduate students of different ages (22,24, 33 years old) 1 male and 2 females. All participants showed sufficient compliance with the HRV control variables (not taking medicines, doing low level of physical activity and not drinking coffee before HRV analysis).

In order to check aromatherapy effects over arousal and concentration, we analyzed the effects of inhaling *sweet basil* and *Ylang ylang* oils on HRV through sessions 1, 2, 3 and 4.

In order to confirm if inhalation aroma is useful and have different effect depending on the period. we analysed HRV parameters in four periods for every session:

1) PRE: 5 min with the participants at rest before starting aroma inhalation.

2) During 1: 5 min at 20 to 25 min after starting aroma.

3) During 2: 5 min at 30 to 35 min after starting aroma.

4) POS: 5 min with the participants at rest at the end of the session

Table 3. Mean and SD of HRV parameters for the 3 participants and for each of the 4 sessions (2 sessions for CO and 2 for OBL).

Aroma	Session	RR £mean	SDNN	RMSSD	LF	HF	LF/HF	LF%	HF%
OBL	Session1								
	PRE	983.04±181.26	148.73±52.62	143.06±45.40	86.37±106.83	117.71±100.62	1.40±0.86	53.64 ±13.31	46.35± 13.31
	During 1	1060.61±76.85	109.09±12.71	119.4±6.28	35.38 ±7.95	42.32 ±11.21	0.9± 0.46	45.83 ±11.73	54.15± 11.73
	During 2	1024.59±70.57	82.16±11.25	97.38±37.14	16.88± 6.06	36.08 ±24.17	0.81±0.79	38.64 ±22.35	61.35± 22.35
	POS	116.18±4.13	116.86±13.93	103.53±19.28	9.55± 4.7	39.87 ±15.08	1.43±1.36	50.37± 27.7	50.27± 28.12
	Session2								
	PRE	927.29± 249.28	131.76± 36.43	94.47 ±22.48	37.48 ±9.77	40.74 ±20.68	1.29 ±1.17	49.74± 19.86	50.25± 19.85
	During1	862.53± 369.85	98.35 ±6.32	97.63± 26.75	26.73 ±1.91	38.53 ±10.46	0.72± 0.21	41.65 ±6.85	58.33 ±6.84
	During 2	1029.97 ±146.52	94.59 ±11.20	91.7± 44.01	23.61± 12.30	28.33 ±19.15	2.25 ±3.16	48.38 ±32.36	51.61± 32.36
	POS	1007.38 ±132.79	137.13± 21.66	96.09± 34.17	22.88 ±8.68	46.92 ±22.32	3.40± 2.62	67.25± 28.72	33.73± 28.72
CO	Session3								
	PRE	1023.65 ±146.44	122.45± 45.53	102.27± 54.20	45.07 ±27.15	47.43± 36.19	1.36± 0.82	54.71 ±13.14	45.27± 13.14
	During1	1027.12± 106.61	125.05± 56.31	112.11± 67.73	45.22± 41.04	42.3 ±30.76	2.77± 1.22	71.26 ±10.66	28.72 ±10.67
	During 2	993.21 ±151.53	127.34± 47.48	105.49± 61.34	32.83± 24.82	43.44± 37.09	3.07± 2.12	68.31± 21.15	31.67 ±21.14
	POS	1022.43 ±114.52	123.63± 52.44	106.60± 52.65	34.66 ±22.46	40.87± 29.02	2.55± 2.01	66.07 ±17.79	34.58 ±18.26
	Session4								
	PRE	1073.96± 146.43	87.11± 3.44	78.74± 18.66	22.09± 6.87	22.9 ±7.3	1.08± 0.56	49.16 ±15.56	50.83± 15.56
	During1	1066.30± 228.45	135.8± 53.94	116.57± 19.45	47.14± 65.08	37.78± 13.95	2.12± 1.81	55.03± 34.77	44.95 ±34.77
	During 2	1069.49± 162.14	133.93± 44.56	126.79 ±29.51	27.23± 5.19	39.49 ±13.13	1.95± 1.75	58.89± 18.24	41.09 ±18.25
	POS	1116.67± 199.09	115.02 ±28.02	78.74 ±18.66	45.42 ±33.64	40.04 ±16.89	1.58± 1.43	51.52 ±30.29	49.13± 30.69

HRV parameters were calculated in four 5min-periods for each session: PRE, 20-25min (During1); 30-35min (During2) and POST. OBL: *Sweet basil*; CO: *Ylang ylang*; HRV: heart rate variability; HR: heart rate; SDRR: standard deviation of all RR intervals; RMSSD: root mean square of differences of successive RR intervals; LF: low frequency; HF: high frequency; PRE: previous period; POS: after inhalation period; LF/HF: LF/HF ratio; LF%: LF values expressed in normalized units; HF%: HF values expressed in normalized units.

Table 3 shows the HRV parameters at baseline (PRE, before inhalation), during the inhalation at 20–25min and at 30-35min, and after the inhalation (POS, for each of the 4 sessions (2 sessions for CO and 2 for OBL). The differences were at baseline (PRE), 20–25min, 30-35min and POST for the following HRV spectral parameters: LF, such that *sweet basil* showed a larger decrease between PRE and 20–25 min than *Ylang ylang* did, and *Ylang ylang* showed a larger LF increase between 20–25 min and POS than *sweet basil* did; RMSSD, such that *sweet basil* showed a larger RMSSD decrease between 20–25 min and POS than *Ylang ylang* did, and *Ylang ylang* showed an RMSSD increase at this stage (see Table 3). We observe a tendency to significance for HFnu and LFnu. There were no significant differences between the two aromas for the ratio LF/HF and RRmean.

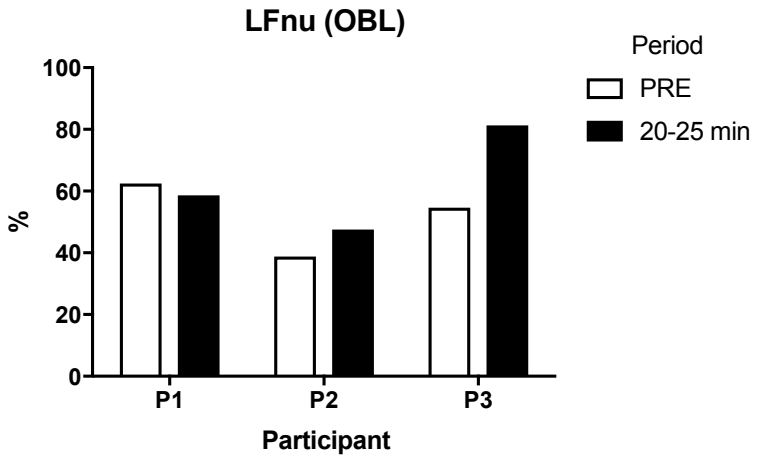


Figure 9. Mean of LFnu (%) parameter for the 3 participants and 2 sessions comparing the periods PRE (before inhalation) and 20-25min after the inhalation of *sweet basil* (OBL; Sessions 1 and 2).

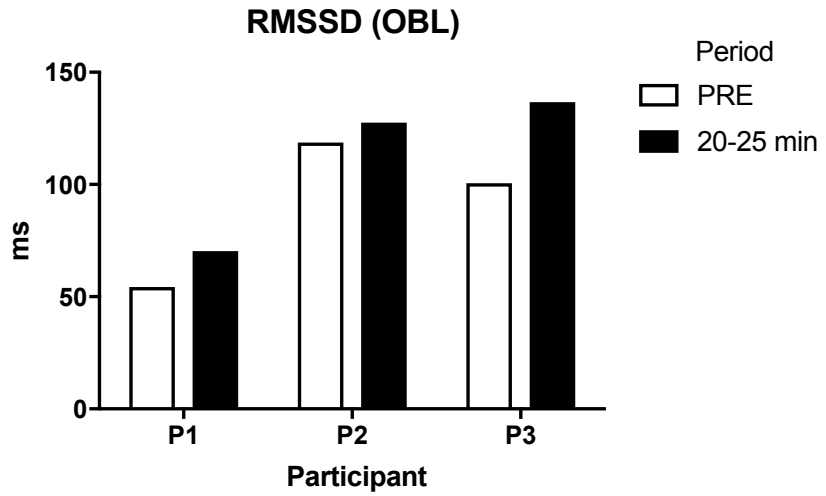


Figure 10. Mean of RMSSD parameter for the 3 participants and 2 sessions comparing the periods PRE (before inhalation) and 20-25min after the inhalation of *sweet basil* (OBL; Sessions 1 and 2).

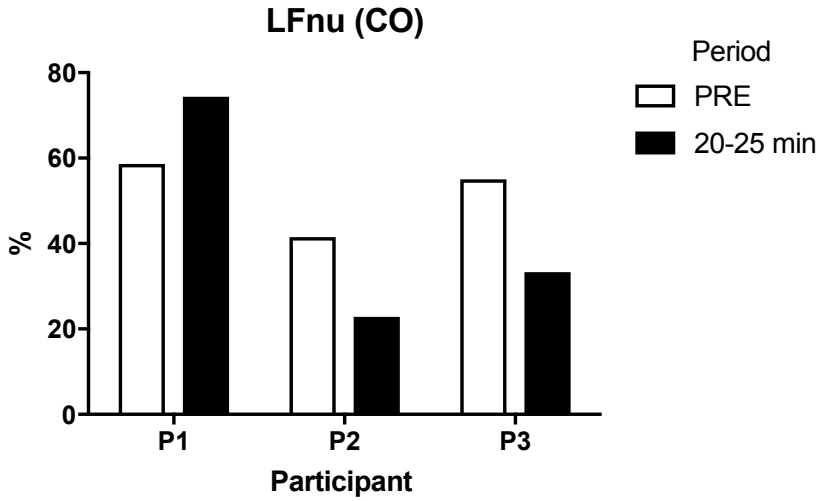


Figure 11. Mean of LFnu (%) parameter for the 3 participants and 2 sessions comparing the periods PRE (before inhalation) and 20-25min after the inhalation of *Ylang ylang* (CO); Sessions 3 and 4).

Figure 9 and Figure 10 show a change of LFnu and RMSSD at 20-25 min (During 1) after starting the *sweet basil* inhalation from baseline (PRE). The values of these parameters increased at 20–25 min from PRE. Figure 11 show a change of LFnu at 20-25 min (During 1) after starting the *Ylang ylang* inhalation from baseline (PRE). The values of these parameters increased at 20–25 min from PRE. In both cases, the peaks were at 20-25min.

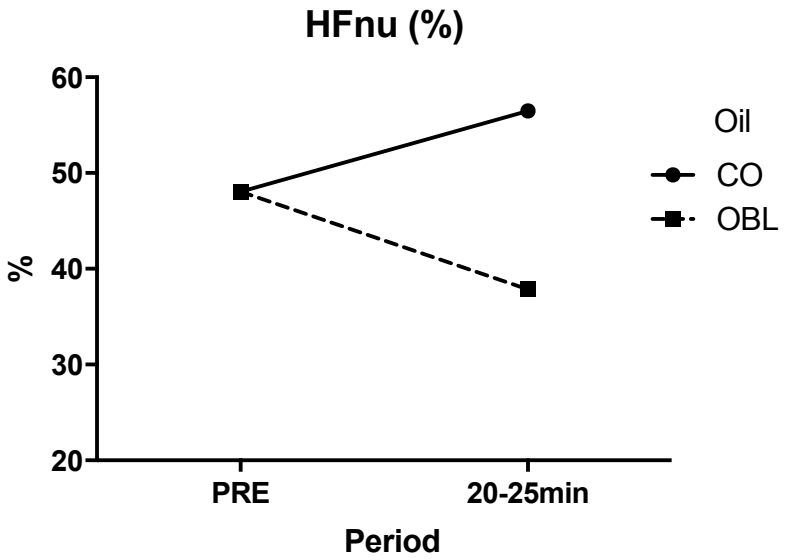


Figure 12. Mean of HFnu (%) parameter for the 3 participants and for the 2 essential oils (OBL: *sweet basil*; Sessions 1 and 2; CO: *Ylang ylang*; Sessions 3 and 4) comparing the periods PRE and 20-25min.

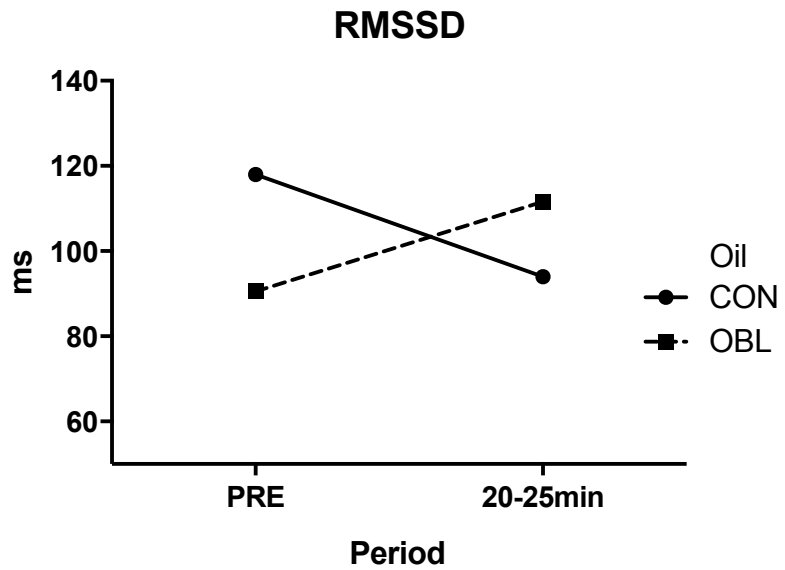


Figure 13. Mean of RMSSD parameter for the 3 participants and for the 2 essential oils (OBL: *sweet basil*; Sessions 1 and 2; CO: *Ylang ylang*; Sessions 3 and 4) comparing the periods PRE and 20-25min.

Figure 12 and 13 show HFnu(%) and RMSSD at baseline (PRE) and During 1 (20-25min) comparing the 2 essential oils (*sweet basil* in sessions 1 and 2; and *Ylang ylang* in sessions 3 and 4). Figure 12 shows a different inhalation effect on HFnu(%) comparing *Sweet basil* and *Ylang ylang* in baseline and 20-25min. For HFnu% parameters we observed an bigger increase at 20-25min inhaling *Ylang ylang* oil. And a

deeper decrease in 20-25min inhaling *sweet basil* oil. That means inhaling *Ylang ylang* oil more affect parasympathetic nervous system. Figure 13 shows a different inhalation effect on RMSSD comparing *Sweet basil* and *Ylang ylang* in baseline and 20-25min.

We found that RMSSD was increases when inhaling *sweet basil* (OBL) and decreases inhaling *Ylang ylang* (CO) at 20-25min comparing PRE. that means inhaling *Ylang ylang* the participants are tonifying and stimulant and inhaling *sweet basil* the participants are relaxant and calmative at that time . That means inhaling *Sweet basil* oil the participants trend to increase their heart variability.

4.6 Discussion

The pilot study tried to set the best conditions to evaluate the effects on HRV of *Ylang ylang* and *Sweet basil* oil through acupuncture point massage, oil inhalation or the combination of both techniques. We wanted to know if HRV analysis is a useful method to explain the effect of aroma inhalation on human sympathetic/ parasympathetic autonomic balance. We employed time and frequency domains to calculate HRV parameters as used in previous studies.

We studied the combination of reducing the anxiety level and increasing the attention and cognitive function with the aid of the HRV analysis among university students.

In our study, we have chosen very common situations in which students usually find themselves during a normal study day at university, such as typing on their computer, reading, writing, etc.

Our data shows a greater improve on HFnu% and RMSSD HRV parameter on

Sweet basil and *Ylang ylang* oil stimulations at 20-25 min after starting inhaling. However, when we compared *Sweet basil* and *Ylang ylang* oil, we found that when inhaling the Sweet basil oil, the RMSSD increased more than when inhaling *Ylang ylang* oil at 20-25min. When inhaling *Ylang ylang* oil, HFnu% increased more than when inhaling *Sweet basil* oil at 20-25 min. When we compared the RMSSD with *Sweet basil* and *Ylang ylang* oil after aroma stimulations at 20-25 min, *Sweet basil* oil showed a higher increase respect *Ylang ylang*. When inhaling *Sweet basil* oil, the participants' attention and cognitive function increased but RMSSD also increased, which means that the participants were more relaxed and calm.

We also analysed the cognitive levels of anxiety and mood state as stress symptoms in our study, but we did not find significant differences among the participants.

We found a clinically meaningful change in HRV-parameter related to anxiety. Participants felt less anxious in the presence of *Sweet basil* and *Ylang ylang* oil. In other words, the level of anxiety of the participants was lower after inhaling *Sweet basil* and *Ylang ylang* oil.

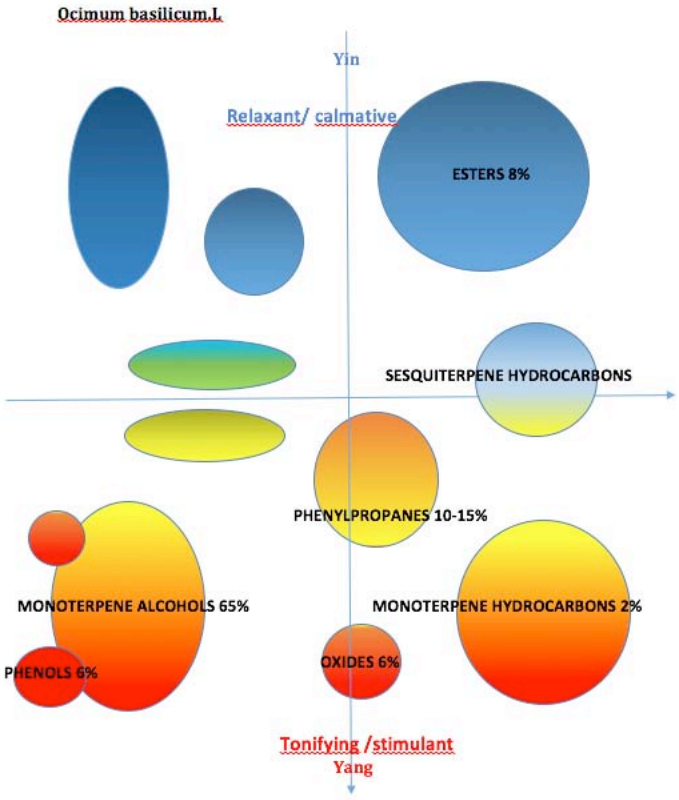
We also found that the highest HRV parameter changed at 20-25min after inhaling the aroma. In this sense, Kuroda et al. found that HF power increased more significantly at 21 min after a 6 min olfactory stimulation of linalool (Matsumoto, Asakura, Hayashi ,2013). In the same line, we found changes at 20-25 min after starting the smelling period. This finding is also consistent with the agitation results previously described.

The data showed that aromatherapy would be effective in promoting parasympathetic activation and heart rate. Therefore, aromatherapy may be useful to

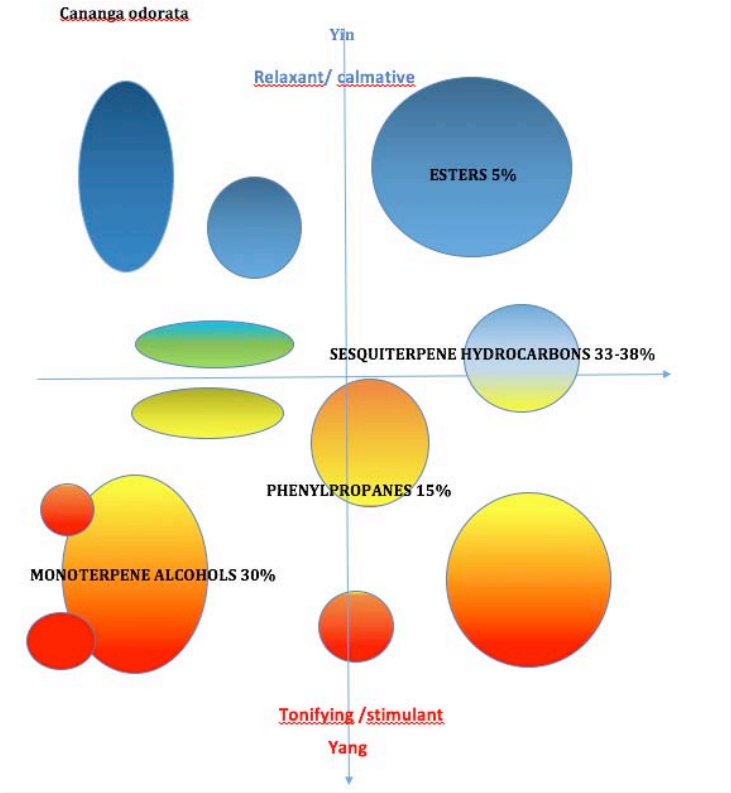
provide quality of study cognitive function and to reduce study anxiety of university students. Similar to previous research reporting beneficial changes in cognition after aroma exposure, the current findings of protective aroma effects by reducing anxiety. This is another evidence that some aromas may protect cognitive functions.

This change could be explained by the combined action of aroma main components (Franchomme, Jollois, penoel,2001). On the one hand, some of these components have sedative effects, such as Esters, Sesquiterpene Hydrocarbons, linalyl acetate and linalool, which could induce changes on the parasympathetic activity (Haze, Sakai and Gozu,2002. On the other hand, methyl chavicol is another component in aroma that could have some activation effects by stimulating the sympathetic activity, as shown in Figure14). The main components of *Sweet basil* oil are methyl chavicol (64.9%) and linalool (25.6%).(see figure 14) The main components of *Ylang ylang* oil are Esters(5%), Sesquiterpene Hydrocarbons(33-38%), Phenylpropanes (15%) and monoterpene alcohols 30% (Franchomme, Jollois, penoel,2001). (See figure 15) The main chemical composition of *Sweet basil* and *Ylang ylang* oil are different the effect for the participants is different. The result in this study is generally consistent with previous studies, which showed that the sympathetic and parasympathetic nervous system activity of the participants was stronger after inhaling the aroma.

Overall, our study agrees with the importance of aroma as a way to improve attention control in a university environment and to reduce study anxiety of university students.



Figuer 14: Ocimum basilicum main chemical composition



Figuer 15. Cananga odorata main chemical composition

We also analysed the use of a massage on an acupuncture point with aroma inhalation together, but it is not clear what kind of intervention works among the

participants. We analysed the data in sessions 1 and 2, with *Sweet basil* inhalation, and sessions 3 and 4, with *Ylang ylang* inhalation. We found that when we used the two methods in the same session, it was not clear which method gave better results.

In this pilot study we have found that both *Sweet basil* and *Ylang ylang* oil supposed a clinically meaningful change. But *Sweet basil* increased students' attention and cognitive function more than *Ylang ylang* oil. Therefore, in our future study (Study 1), we will use *Sweet basil* oil.

4.7 Conclusions

Our study confirms that the use of aroma inhalation *Sweet basil* and *Ylang ylang* oil together with a massage on an acupuncture point can reduce the anxiety level and increase the attention and cognitive function of university students. We have compared *Sweet basil* and *Ylang ylang* oil and we found that *Ylang ylang* oil have sedative effects, which could induce changes on the parasympathetic activity and reduce anxiety at the same time. *Sweet basil* oil could have some activation effects by stimulating the sympathetic activity and reduce anxiety at the same time. Both oils have shown changes when we compared the PRE and POST inhalation aroma. However, our study focuses on increasing the attention and cognitive function and reducing the anxiety level of university students. Thus, this study has ensured that it will be better to use *Sweet basil* oil in our next study.

We have done a qualitative research in our study. After the interview, we found that participants felt uncomfortable when massaging an acupuncture point by themselves, but in our data the acupuncture massage was not significant. Therefore, in our future study, we will not use acupuncture massage.

5. STUDY 1: (Aroma improve attention control in university students).

5.1 Objective

The aim of the study is to determine if aromatherapy reduces anxiety and improves study concentration of university students.

5.2 Sample

The initial sample was composed by 21 healthy undergraduate students (age between 18 and 33 years old; gender: 7 males and 14 females). They were recruited from a university school through publicity and registration. First, all participants completed a standardized health questionnaire in a previous collective session in order to accomplish the following inclusion criteria: 1) age between 18 and 33; 2) No clinical diagnosis of autonomic dysfunction; 3) no clinical diagnosis of respiratory, cardiovascular, endocrine or systemic disorder; 4) no olfactory function problems. Participants agreed to participate voluntarily in the study and were randomly assigned to the experimental and control group. The final sample, which completed all sessions of the study was composed by 12 students, 7 participants in the experimental group and 5 participants in the control group. We did not inform participants about what kind of fragrance we would use for the sessions. All participants were volunteers and provided informed consent to participate in the study. The protocol was reviewed and approved by the local ethics committee.

5.3 Instruments

For the study, we used the following instruments, which are explained in part 3.3 above. (common instruments for all studies).

- Questionnaires: STAI, POMS, SF12 and *Face* Scale
- Oils and diffusers for inhaling: *Sweet basil* (OBL) for EXP and *Sweet almond(*PAV) for CON.
- HRV analysis using iPad, chest bands and FitLab system.

5.4 Procedure

In order to distinguish the specific effects of aromatherapy reducing anxiety and improving quality of study in university students we conducted this study with 4 sessions for all participants. Each group attended 3 sessions separately in different days (CON and EXP groups had to inhale different oils scattered in the lab) and a final session at the exam day. Thus, session 1 to session 3 were performed in different days for each group between 9:30AM and 11:00 AM in a lab situation. Session 4 was performed between 9:30AM and 11:00AM in two separate classrooms (one for each group) during a postgraduate exam at the same time. We controlled the ambient temperature for all sessions between 22 ºC and 25 ºC. The intervention consists on inhaling essential oil for EXP and vegetable oil for CON during 50 min in sessions 1 to 3, and during 90 min in session 4. (See Table 4)

Table 4: Sessions characteristics for Study 1.

Session	Participants	Time	Place	Oil	Use way	Homework	Group
Session 1	7	60min	lab	OBL	Smell	no	EXP
Session 1	5	60min	lab	PAV	Smell	no	CON
Session 2	7	60min	lab	OBL	Smell	yes	EXP
Session 2	5	60min	lab	PAV	Smell	yes	CON
Session 3	7	60min	lab	OBL	smell	yes	EXP
Session 3	5	60min	lab	PAV	smell	yes	CON
Session 4	12	90min	Classroom1 Classroom2	OBL PAV	smell	no	EXP CON

5.4.1 Procedure for Sessions 1 to 3

In sessions 1 to 3, which took place in the lab, the participants were asked to breathe normally during around 60 minutes in the lab while sitting around a shared table. They were asked not to do any vigorous physical activity, just the normal things that they usually did in the university classroom, such as reading, writing or using a computer. The oil diffusers were scattered all over the room. All participants were asked not to consume any food or beverages containing alcohol or caffeine after the evening of the day preceding the experiment. They were also asked not to do vigorous physical activity during 24 hours before the lab sessions. Firstly, participants were required to fill out the PRE questionnaires (STAI-State, POMS and the Feeling scale). Secondly, participants were asked to wear the cardiac chest band (Bluetooth connected) and rest in a sitting position in front of the table. After that, HRV-PRE was recorded for 5 minutes with the participants at this rest situation (sitting, with their eyes open, hands on their knees, doing no activity, breathing naturally and without speaking). In the fourth place, the participants inhaled *Sweet basil* (EXP) or *Sweet almond* (CON) thanks to the aroma launched by the diffusers. It allowed a proper smelling throughout the room around 50min and HRV was recorded during all this time. Then, a 5min HRV-POStest was recorded (sitting, with their eyes open, hands on their knees, doing no activity, breathing naturally and without speaking). After that, participants were asked to fill out the POS questionnaires (STAI-State, POMS and the Feeling scale). Then, participants were asked to take an aroma necklace (*Sweet basil /Sweet almond*) for inhaling *Sweet basil* (EXP) or *Sweet almond* (CON) during 3 hours per day at home. (Session 1), Then, an interview about participant's feeling related to

the aroma at home was performed (Sessions 2 and 3).

5.4.2 Procedure for Sessions 2

The general procedure for Session 2 was the same than for sessions 1 and 3, but we performed HRV analysis continuously all along the session. We calculated the HRV parameters before starting inhalation (baseline) and after starting it in periods of 5 min at 20-25 min and 30-35 min.

5.4.3 Procedure for Session 4.

Participants arrived to the lab on the exam day in the morning. The 3 first steps were the same than in the previous sessions. But in the fourth step, participants went to a different classroom for CON and EXP, mixed with other students who also did the same exam and wearing the cardiac chest band. In the classroom 1 EXP participants inhaled Sweet basil aroma from the diffusers which allowed a proper smelling all over the classroom. CON participants inhaled Sweet almond aroma with similar conditions. HRV was recorded during all the exam time (around 90 min). After participants finished their exam, participants were required to perform the HRV-POStest in the same exam classroom (sitting, eyes open, hands on their knees, doing no activity, breathing naturally and without speaking). Finally, participants were asked to fill out the POS questionnaires (STAI-State, POMS and the Feeling scale).

5.5 Results

5.5.1 Results for Study 1 (4 sessions)

Seven participants completed de study in the EXP group and five in the CON group. Nine of the 21 initial participants did not complete the study because they

could not attend 50% of the lab sessions. Table 5 shows the defining characteristics of participants. There were no significant differences between the two groups in terms of gender, age, physical activity level, anxiety trait (STAI-T), health perception (SF12) and academic performance. Likewise, no significant differences between the two groups were found in terms of HRV control variables, such as taking medicines, doing physical activity and drinking coffee. Thus, these results ensure that there were equal characteristics among the participants in both groups.

Table 5. Participants characteristics for Study 1: gender (%), age, initial questionnaires and physical activity level for the two groups.

VARIABLE	CON (n=5)	EXP (n=7)	*p* value
Gender (n)			
Females	4	4	
Males	1	3	NS
Age(years)	24.00±4.69	22.29±1.25	NS
Exam result	8.04± 0.97	7.24± 1.09	NS
STAI-T	18.67±3.80	16.57±10.40	NS
STAI-S	24.40± 7.77	14.58± 6.18	NS
PCS (SF12)	49.48±10.07	52.56±3.18	NS
MCS (SF12)	35.71±8.83	41.95±6.35	NS
Face	6.00 ±1.63	7.57± 1.13	NS
Tension (POMS)	5.44± 2.81	2.28±1.56	NS
Hostility (POMS)	0.78 ±0.50	0.23 ±0.49	NS
Fatigue (POMS)	3.88± 1.63	2.66± 1.40	NS
Depression (POMS)	1.83 ±1.16	0.67 ±0.96	NS
Vigour (POMS)	5.77± 1.31	6.33 ±1.66	NS
POMS Total	5.54 ±0.66	4.43± 0.53	NS
Physical Activity			
No active	80%(4)	71.4%(5)	NS
Active	20%(1)	28.6%(2)	

STAI-T: Trait scale of the State-Trait Anxiety Inventory; **STAI-S**: State scale of the State-Trait Anxiety Inventory; **POMS**: Profile of Mood States; **EXP**: experimental group; **CON**: control group; **PCS**: Physical Component (SF12); **MCS**: Mental Componente (SF12); **SF-12**: the 12-item Short Form Health Survey;

5.5.2 LONG TERM RESULTS: Aromatherapy effects through 4 sessions.

In this part we will present the results to explain the AR effects through the four sessions of the Study. Table 6 and Figures 16 and 17 show SF12 scores (Means and SD) in the 4 sessions for both groups (EXP and CON). GLM analysis, which compares the evolution through the 4 sessions, show significant differences between both groups. Therefore, on the one hand, EXP increases PCS in Sessions 3 and 4, while CON decreases PCS through the sessions ($F_{(3)} = 4.37$; $p = 0.01$) (see Figure 16). On the other hand, CON increases MCS in Sessions 3 and 4, meanwhile EXP maintains the same MCS scores through the sessions ($F_{(3)} = 3.44$; $p = 0.03$) (see Figure 17).

In the case of the Physical Component (PCS) of SF12, ONEWAY analysis for each session shows that the only difference (with tendency to significance) between groups is in Session 3. In this session, EXP shows a bigger PCS than CON ($F_{(9,1)} = 3.66$; $p = 0.08$). There are no significant differences between the sessions for all other SF12 scores including MCS component.

Table 6. SF12 scores (Means and SD) in the 4 sessions of Study 1 for both groups.

QUEST	GROUP	Session 1	Session 2	Session 3	Session 4
PCS_SF12	CON	56.80±6.92	56.50±2.17	**54.62± 3.05**	53.96± 4.83
	EXP	56.64±5.27	53.87±5.82	**58.62± 3.74***	57.63± 4.47
MCS_SF12	CON	43.17± 8.85	48.86 ±7.44	54.62± 3.88	53.80± 7.70
	EXP	48.14±12.72	50.67± 7.84	47.39± 11.39	48.51± 8.64

*EXP > CON ($F_{(9,1)}$= 3.66; p = 0.08). No significance for all other SF12 scores. **PCS**: Physical Componente;

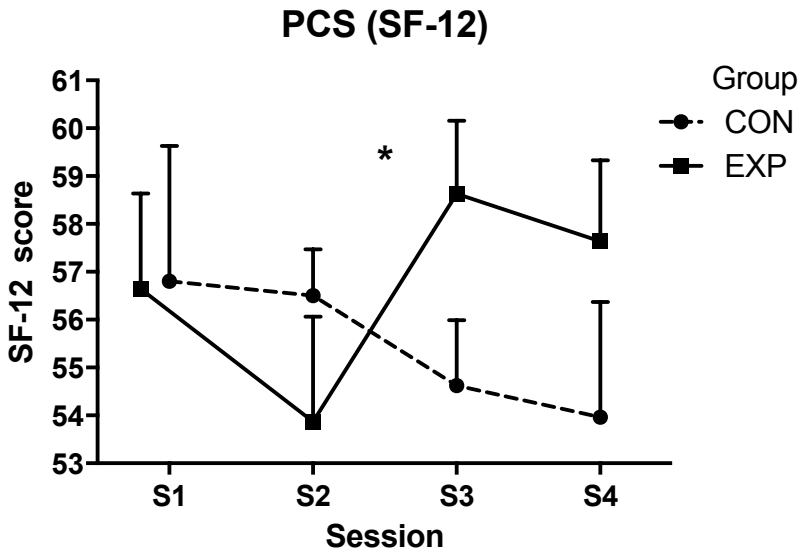


Figure16. PCS (SF12) scores (Means and SD) in the 4 sessions for both groups.

* EXP: significant increase, p=0.01 (GLM).

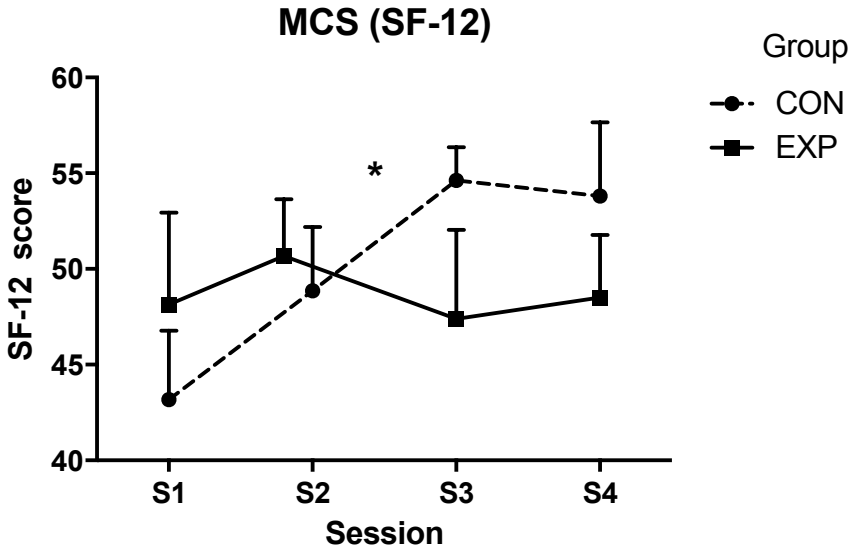


Figure 17. MCS (SF12) scores (Means and SD) in the 4 sessions for both groups.

* CON: significant increase, p=0.03 (GLM).

Table 7 shows the anxiety (SAI) and global feeling (FACE) scores (Means and SD) in the 4 sessions of both groups and in both situations, before (PRE) and after (POS) sessions. ONEWAY analysis for each session shows that he only significant difference between groups is before Session 4. In this session, EXP shows a bigger FACE_PRE score than CON ($F_{(9,1)}$ = 8.18; p = 0.01). There are no significant differences between groups into every session for all other PRE and POS scores, before and after sessions.

Table 7. STAI-S and *Feeling* scale scores (Means and SD) in the 4 sessions of Study 1 for both groups.

QUEST	GROUP	Session 1	Session 2	Session 3	Session 4
STAI-S_PRE	CON	15.50±8.73	15.40±5.32	13.00±4.30	23.25±4.79
	EXP	16.14±10.07	19.71±11.32	9.33±6.19	25.28±3.04
STAI-S_POS	CON	14.00±10.27	14.6± 3.64	9.60±7.89	23.00±3.91
	EXP	9.43±4,35	13.14 ±6.84	6.83±3.54	24.00±2.7
Feeling_PRE	CON	7.33±1,03	7.40±0.89	5.60±3.21	**6.00±0.82**
	EXP	7,00±1,15	7.00 ±1.29	7.83±1.60	**7.43±0.79***
Feeling_POS	CON	7.50±0.83	6.40±2.50	7.60±1.14	7.00±0.81
	EXP	7.43 0.78	7.43±1.27	8.00±1.55	7.71±1.4

*EXP > CON ($F_{(9,1)}$ = 8.18; p = 0.01); STAI-S: State scale of the State-Trait Anxiety Inventory; PRE: previous period; POS: after inhalation period.

Table 8 shows the POMS scores (Means and SD) in the 4 sessions for both groups and for both situations, before (PRE) and after (POS) sessions. ONEWAY analysis of each session shows significant difference between the groups in the *Tension* score before Session 3 (PRE: CON > EXP; $F_{(9,1)}$ = 5.41; p = 0.045); in the *Fatigue* score before Session 3 (PRE: CON > EXP; $F_{(9,1)}$ = 7.41; p = 0.023); in the *Fatigue* score after Session 3 (POS: CON > EXP; $F_{(9,1)}$ = 5.10; p = 0.050); for POMS TOTAL score before Session 3 (PRE: CON > EXP; $F_{(9,1)}$ = 10.72; p = 0.010). There is also a trend towards significance in the *FATIGUE* score after Session 2 (POS: CON > EXP; $F_{(10,1)}$ = 3.62; p = 0.086); in the *FATIGUE* score before Session 4 (PRE: CON > EXP; $F_{(9,1)}$ = 3.69; p = 0.087); in the *VIGOUR* score before Session 4 (PRE: EXP > CON; $F_{(9,1)}$ = 3.71; p = 0.086). There are no significant differences between in all the other PRE and POS scores, before and after sessions.

Table 8. POMS scores (Means and SD) in the 4 sessions of Study 1 for both groups.

POMS scales	GROUP	Session 1	Session 2	Session 3	Session 4
TENSION_PRE	CON	2.28±2.16	2.13±2.66	**2.66±1.13**	4.75±3.84
	EXP	2.24±2.16	3.52±2.89	**0.50±0.78***	4.52 ±2.69
TENSION_POS	CON	0.89 ±1.42	1.80 ± 2.26	1.06 ±1.68	1.91 ±1.16
	EXP	0.33±0.57	1.86±1.82	0.27± 0.44	1.95±2.66
HOSTIL_PRE	CON	0.22± 0.40	0.33±0.47	0.33±0.58	0.08± 0.17
	EXP	0.29±0.75	0.62±1.64	0.22 0.40	0.43±0.99
HOSTIL_POS	CON	0.11±0.17			0.33± 0.57
	EXP	0.95±0.25	0.09±0.25		0.57±0.99
FATIGUE_PRE	CON	2.56 ±1.66	3.40±1.09	2.90±1.80	**3.75±1.32**
	EXP	1.81±1.42	2.33±2.97	0.72 ±0.80	**1.19±2.43****
FATIGUE_POS	CON	1.5±1.24	**3.13±1.99**	**1.60 ± 1.47**	3.42 ±0.83
	EXP	0.62±0.78	**1.24±1.47****	**0.22±0.27***	1.14±2.88
DEPRES_PRE	CON	0.90 ±0.57	0.33 ± 0.57	0.60 ±0.54	1.58±1.54
	EXP	1.33±2.82	1.14±2.04	1.05 ± 1.25	0.86±1.99
DEPRES_POS	CON	0.56±0.75	0.80 ± 1.26	0.26± 0.43	0.33± 0.38
	EXP	O.38±0.73	0.67±1.62	0.27± 0.53	0.67±1.27
VIGOUR_PRE	CON	6.50±1.05	5.80±1.87	5.73± 2.06	**4.83±1.89**
	EXP	5.95±1.93	5.38±2.14	5.83±1.85	**6.5±1.06****
VIGOUR_POS	CON	5.56± 2.56	5.73±1.80	6.13±2.98	4.83±1.89
	EXP	5.42±1.53	5.47±2.21	6.00±2.33	6.52±1.06
TOTAL_PRE	CON	4.50 ±7.76	4.36±1.01	**4.45±0.52**	5.00±1.31
	EXP	4.32±1.04	4.72 ±1.56	**3.36±0.24***	4.70 ±1.47
TOTAL_POS	CON	3.70±0.54	4.29 ±1.28	3.81± 0.54	4.11±0.64
	EXP	3.37±0.31	3.86±0.75	3.35±0.32	4.09±1.33

*p<.05; **p>.05 and p<.09). PRE: previous period; POS: after inhalation period.

We calculated the difference between the before and the after sessions for questionnaire scores to compare the evolution between groups through the four sessions. Table 9 shows the differences (Means and SD) between PRE and POS scores (PRE - POS) in all questionnaires in the 4 sessions, for both groups. ONEWAY analysis for each session shows a clear *tendency* to *significance* for STAI-S difference score (DIF_STAI-S) in session 2(see the Figure 18): EXP > CON ($F_{(10,1)} = 4.37$; $p = 0.063$). There is no significance for all other questionnaire scores.

Table 9. Differences (Means and SD) between PRE and POS scores (PRE - POS) of all questionnaires in the 4 sessions for both groups.

QUESTIONNAIRES	GROUP	Session 1	Session 2	Session 3	Session 4
DIF_STAI-S	CON	1.50±12.01	0.80±3.11	3.4±4.09	0.25± 3.40
	EXP	6.71±6.62	6.57±5.53	2.5±3.6	1.28 ±1.89
DIF_FACE	CON	-0.17±0.40	1.00 ±2.83	-2.00 ±3.93	-1.00± 0.81
	EXP	-0.43±0.79	-0.43 ±0.79	-0.17± 0.40	-0.29±1.25
DIF_TENSION	CON	1.39 ±0.88	0.33±1.17	1.6 ±2.07	2.83±2.98
	EXP	1.90±1.67	1.6 ±1.52	0.22±0.34	2.57±1.46
DIF_HOSTIL	CON	0.11±0.27	0.33±0.47	0.33±0.57	-0.22±0.69
	EXP	0.19±0.50	0.52 ±1.39	0.22±0.40	-0.14± 0.69
DIF_FATIGA	CON	1.06 ±1.54	0.26 ±1.09	1.33±1.27	0,33±1.41
	EXP	1.19 ±1.17	1.09±2.02	0.50±0.58	0.05±0.59
DIF_DEPRES	CON	0.38± 0.68	-0.47±1.28	0.33±0.47	1.25±1.52
	EXP	0.95±2.09	0.47±0.53	0.78 ±1.20	0.19± 0.99
DIF_VIGOR	CON	0.94 ±1.81	-0.13 ±0.76	-0.40±2.25	-0.17±1.03
	EXP	0.52 ±0.92	-0.95 ±0.68	-0.17±1.44	0.38±1.07
DIF_TOTAL	CON	0.78±0.59	0.07±0.51	0.64±0.40	0.40±0.61
	EXP	0.95± 0.85	0.86±0.91	0.31±0.30	0.60±0.44

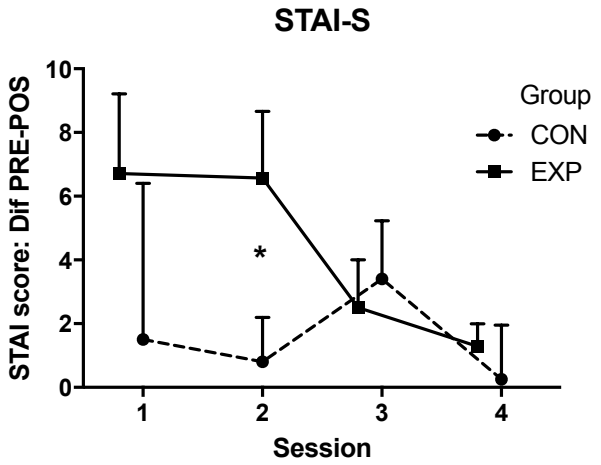


Figure 18. Differences between PRE and POS scores (PRE - POS) for STAI-S questionnaire in the 4 sessions, for both groups.* EXP > CON; p = 0.06 (ONEWAY).

Table 10 shows the HRV parameters (Means and SD) in the 4 sessions for both groups and for both situations, before (PRE) and after (POS) sessions. ONEWAY analysis for each session shows a trend towards significant difference between groups for HRmean after Session3 (POS: CON > EXP; $F_{(10,1)}$ = 4.01; p = 0.073); and for RRmean

after Session3 (POS: EXP > CON; $F_{(10,1)}$ = 3.66; p = 0.085). There are no significant differences between groups in every session for all other HRV parameters, before and after the sessions.

Table 10. HRV parameters (Means and SD) in the 4 sessions for both groups.

HRV	GROUP	Session 1	Session 2	Session 3	Session 4 [a]
HRmean_PRE	CON	75.73±9.19	79.26±10.19	80.33±8.57	
	EXP	69.53±10.43	70.89±11.75	73.13±14.12	79.03±9.34
HRmean_POS	CON	75.77±10.10	78.39±13.65	**80.29±8.00**	
	EXP	80.59±39.67	67.25±11.10	**68.34±11.41****	70.61±8.69
RRmean_PRE	CON	828.72±109.03	795.89±111.23	780.80±95.13	
	EXP	910.97±136.76	901.67±172.05	876.74±172.46	791.46±92.67
RRmean _POS	CON	832.73±131.16	814.13±148.39	**781.51±83.73**	
	EXP	866.68±311.93	803.49±344.17	**934.0±161.86****	890.02±112.19
SDRR_PRE	CON	69.29±16.81	62.76±7.13	65.69±20.69	
	EXP	72.74 23.28	78.63 ±33.07	62.33±32.19	56.14±22.37
SDRR_POS	CON	69.42±8.77	67.39±15.08	74.05±13.83	
	EXP	70.72±19.67	80.36±32.04	82.40±29.07	88.89±34.56
RMSSD_PRE	CON	48.21±15.47	36.94±12.94	46.27±22.80	
	EXP	51.04±20.52	54.75±35.13	49.11±40.26	31.07±13.80
RMSSD _POS	CON	48.49±14.85	43.59±20.53	43.8 ±11.95	
	EXP	53.39±24.76	56.14±33.43	62.87±52.51	50.55±12.58
LFHF_PRE	CON	1.52±1.16	2.57±1.34	2.9±3.39	
	EXP	1.33±1.19	1.70±1.74	1.67±1.38	1.86±0.93
LF/HF_POS	CON	1.86±1.61	3.0 ±3.08	2.71±2.06	
	EXP	1.42±1.06	1.61±1.53	1.55±1.45	2.72±1.55
LFnu_PRE	CON	53.70±19.18	69.37±9.16	59.34±25.66	
	EXP	48.83±21.93	52.01±23.73	52.24±25.81	62.03±11.44
LFnu_ POS	CON	55.83±19.18	63.33±20.88	64.77±19.67	
	EXP	49.95±24.54	50.05±25.77	50.59±24.27	68.57±14.32

(**p>.05 and p<.09; [a] means for CON were not calculated because there were only 2 participants for this group in Session 4).

We calculated the difference between the results before and after the sessions for HRV parameters to compare the evolution between groups through the four sessions. Table 11 shows the differences (Means and SD) between PRE and POS parameters (PRE-POS for HRmean; and POS-PRE for all other parameters) in the 4

sessions, for both groups. ONEWAY analysis for each session shows a clear *tendency* to *significance* for the RRmean difference POS-PRE (DIF_RRmean) in Session 3: EXP > CON ($F_{(10,1)}$ = 3.94; p = 0.075)(See figure 19) .There is no significant difference for all other HRV parameters.

Table 11. HRV parameters differences (Means and SD) between POS and PRE in the 4 sessions for both groups: PRE-POS for HRmean; and POS-PRE for all other parameters.

HRV Dif	GROUP	Session 1	Session 2	Session 3	Session 4 [a]
DIF_HRmean	CON	-0.40±6.04	0.87±6.41	0.04±1.01	
	EXP	-11.05±40.70	3.64±1.81	4.79±6.25	8.42±7.19
DIF_RRmean	CON	4.01±61.13	18.24±57.19	**0.70±13.3**	
	EXP	-44.28±230.95	-98.19±387.98	**57.26±61.86****	98.56±69.37
DIF_SDRR	CON	0.13±10.57	4.63±12.84	8.36±20.87	
	EXP	-2.02±11.71	1.73±14.31	20.06±20.89	32.74±19.78
DIF_RMSSD	CON	0.28±17.79	6.66±10.18	-2.46±15.19	
	EXP	2.35±4.4.3	1.39±11.26	13.76±23.50	19.48±12.43
DIF_LFHF	CON	0.34±1.01	0.42±1.82	-0.20±3.75	
	EXP	0.09±0.74	-0.09±1.64	-0.12±1.93	0.86±1.96
DIF_LFnu	CON	2.13±15.4	-5.7±14.95	5.43±19.88	
	EXP	1.13±11.02	-1.96±12.87	-1.64±22.25	6.5±19.23

(**p>.05 and p<.09; [a] means for CON were not calculated because there were only 2 participants for this group in Session 4)

Firstly, we analysed the evolution of HRV parameters through the 4 sessions for EXP group with a GLM within-subjects analysis. This analysis found a significant effect for the increasing DIF_SDRR values (POS-PRE) from Session 1 to Session 4 (linear model; (F= 17.84; p = 0.006) (see Figure 20). The same effect was observed on DIF_RMSSD (POS-PRE) from Session 1 to Session 4 (linear model; (F= 13.32; p = 0.011) (see Fig 21). There is no significant difference in all the other HRV parameters.

Secondly, we analysed the evolution of HRV parameters through the 3 sessions for CON group with a GLM within-subjects analysis (Session 4 for CON were not included because there were only 2 participants for this group in this session). There is no significance for any HRV parameters for CON group.

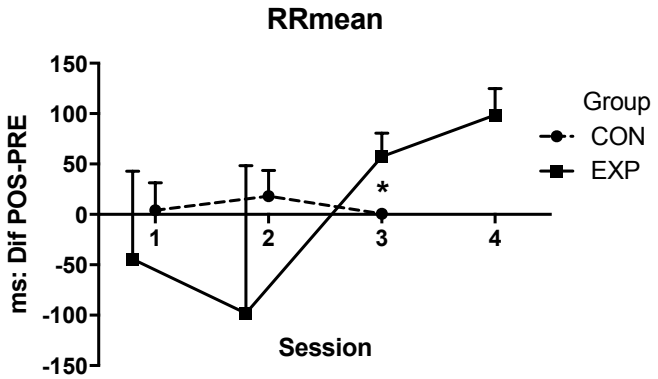


Figure 19. Differences between POS and PRE scores (POS-PRE) for RRmean parameter in the 4 sessions, for both groups.* EXP > CON; p = 0.07 (ONEWAY). (Session 4 for CON is not included because there is only 2 participants).

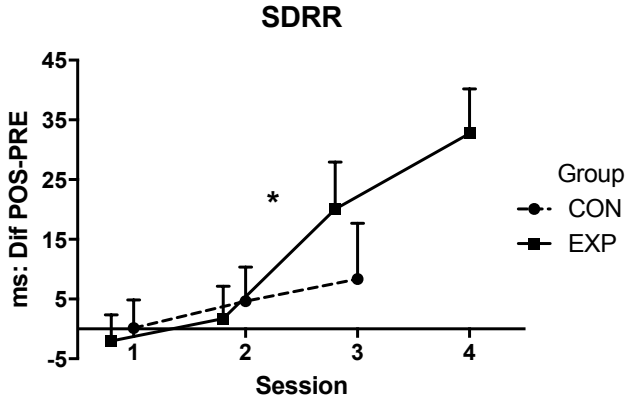


Figure 20. Differences between POS and PRE scores (POS-PRE) for SDRR parameter in the 4 sessions, for both groups.* EXP: significant increase, p=0.006 (GLM). (Session 4 for CON is not included because there is only 2 participants).

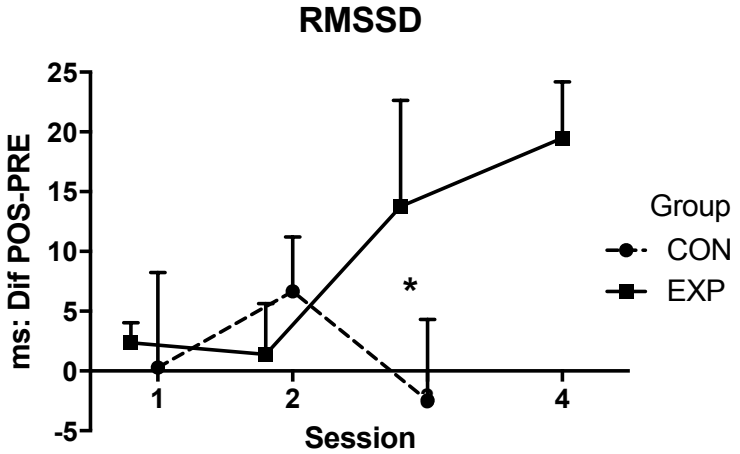


Figure 21. Differences between POS and PRE scores (POS-PRE) for RMSSD parameter in the 4 sessions, for both groups.* EXP: significant increase, p=0.006 (GLM). (Session 4 for CON is not included because there is only 2 participants).

5.5.3 SHORT TERM RESULTS: Aromatherapy effects through one session.

In this part we will present the results to explain the AR effects during the second session of the Study. Seven participants completed de study for the Aromatherapy Group and five for the Control Group. 9 participants did not complete the study because they could not attend the lab session. Table 5 shows the defining characteristics of the participants. There were no significant differences between the two groups in gender, age, physical activity level, anxiety trait (STAI), health perception (SF12) and academic performance. No significant differences between the two groups were found by HRV control variables like taking medicines, doing physical activity and drinking coffee. Thus, these results ensure that the participants of the two groups had equal characteristics.

The Table 12 represents the comparison between the two groups about HRV parameters along the second session of the study, at baseline, at the 10-15 min, at the 20-25 min, at the 30-35 min and POS periods from the starting of smelling period. In this session, the essential oil for EXP was *Sweet basil* and the neutral oil for CON was *Sweet almond.*

Table 12. HRV parameters (mean±SD) for baseline (PRE), 10-15min (Dur 1), 20-25min (Dur 2) and 30-35 min (Dur 3) in the second session of the study, for both groups.

HRV	GROUP	HR	RR mean	SDNN	RMSSD	LF	HF	VLF	LF/HF	LF%	HF%
PRE	CON	76.48±9.86	795.89±111.23	62.77±7.13	37.03±12.98	1527.98±524.31	734.41±419.15	1547.09±628.71	2.6±1.31	69.66±9.03	30.34±9.03
	EXP	68.42±11.45	901.58±172.99	73.84±33.22	50.45±34.47	1811.56±2416.53	1965.89±3341.73	2691.46±3702.61	1.79±1.94	52.21±24.27	47.79±24.27
Dur 1	CON	78.34±10.93	778.62±114.86	76.58±18.51	41.22±15.32	1396.74±348.21	729.21±307.16	1693.54±1033.83	2.35±1.34	65.62±14.61	34.38±14.61
	EXP	70.09±9.80	871.07±126.61	89.03±26.83	48.31±22.16	1982.38±1048.29	841.38±554.01	3365.05±2433.62	2.98±1.76	68.72±17.26	31.28±17.26
Dur 2	CON	75.92±13.65	811.58±149.24	69.33±18.52	**44.24±21.29**	1473.99±753.99	779.34±487.96	**1857.16±1197.74**	3.17±2.72	66.15±20.90	33.85±20.90
	EXP	68.20±9.91	897.49±142.73	105.59±29.42	**58.66±34.11***	3768.36±2810.05	1240.76±1060.55	**7319.76±5498.00***	6.09±6.34	73.37±20.68	26.63±20.68
Dur 3	CON	75.58±13.05	814.24±148.31	67.32±15.19	43.53±20.77	1554.86±745.11	1178.8±890.32	1745.32±1745.37	2.82±2.99	62.77±20.07	37.23±0.06
	EXP	67.29±10.99	914.15±162.58	84.40±28.91	51.88±26.79	1363.71±990.60	1582.06±2039.25	2561.00±1977.86	1.58±1.09	53.61±22.52	46.39±22.52
p(GLM)		NS	NS	0.030	NS	0.021	NS	NS	NS	0.015	0.015

*(P<0.05; ONEWAY)

The ONEWAY analysis of each period shows a significant difference in Session 2 between groups for *SDRR* in the 20-25 min period. In this period the EXP group has a higher heart rate variability than CON (EXP > CON; $F_{(10,1)} = 5.84$; $p = 0.036$). EXP also shows higher VLF values than CON in the same Session 3 (EXP > CON; $F_{(10,1)} = 4.65$; $p = 0.05$). There are no significant differences between groups in any period for all the other HRV parameters.

An analysis of variance according to a general linear model (GLM) shows differences between the two groups in terms of evolution throughout of the four stages of the session (Baseline, 10-15 min, 20-25 min and POS) for some HRV parameters. Thus, the EXP group shows a significant peak at 20-25 min period for *SDRR* ($F_{(10,1)} = 6.35$; $p=0.03$; see Figure 22), LF ($F_{(10,1)} = 7.44$; $p=0.021$; see Figure 23) and LF% ($F_{(10,1)} = 8.63$; $p=0.015$; see Figure 24), in comparison with CG; conversely proportional differences to LF% are observed for HF% ($F_{(10,1)} = 8.63$; $p=0.015$) (see Figure 24). There is no significant difference for all the other HRV parameters and periods.

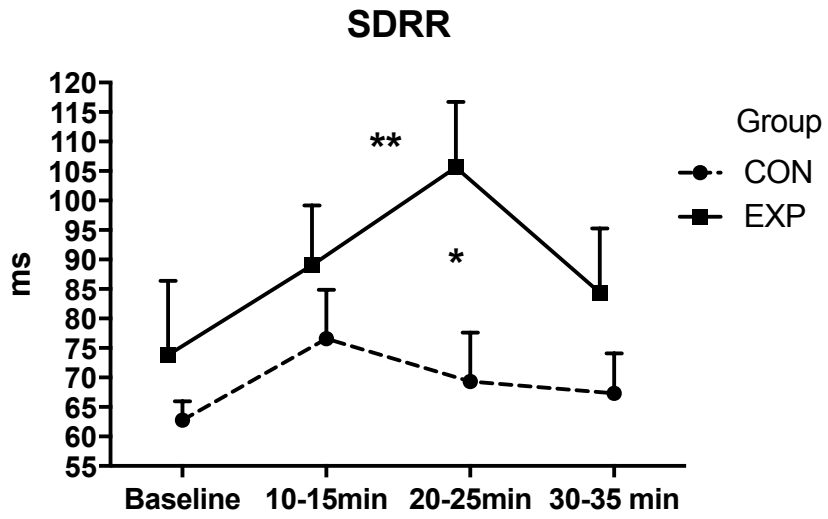


Figure 22. SDRR values (Means and SEM) in the 4 periods along Session 2 for both groups. * EXP > CON; p = 0.036 (ONEWAY). ** EXP: significant peak, p=0.03 (GLM)

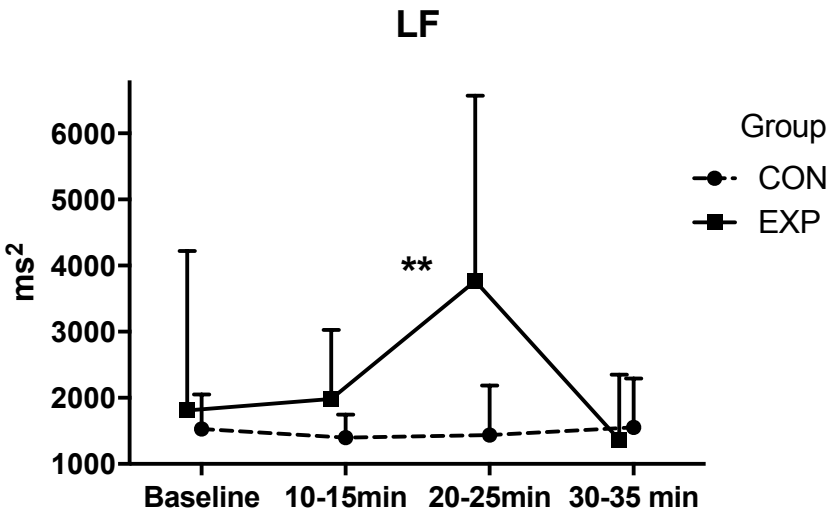


Figure 23. LF values (Means and SEM) in the 4 periods along Session 2 for both groups. ** EXP: significant peak, p=0.021 (GLM)

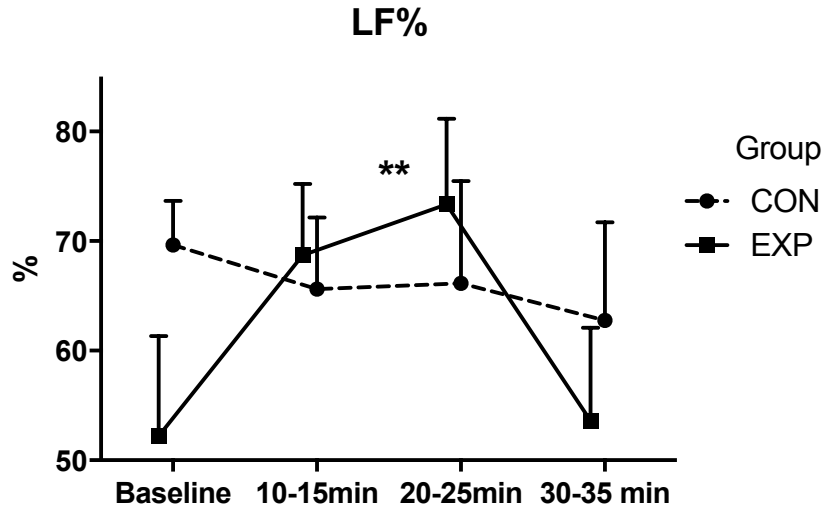


Figure 24. LFnu% values (Means and SEM) in the 4 periods along Session 2 for both groups. ** EXP: significant peak, p=0.015 (GLM).

Table 13 shows the questionnaires scoring for both groups before (PRE) and after (POS) Session 2. We applied GLM analysis to compare the evolution from PRE to POS-session between both groups.

This analysis shows a significant difference between groups for STAI-S scale, indicating than EXP shows a more pronounced decrease in POS than CON ($F_{(10,1)} = 4.37$; $p = 0.05$)(see Figure 25). GLM also shows a tendency to significant difference between groups for *Tension* scale (POMS) likewise ($p = 0.98$) (see Figure 26). EXP group also

shows a more pronounced decrease in POS than CON in the same sense (but without statistical significant differences) for *Depression* scale (POMS) (see Figure 27), ant Total POMS score(see Figure 28).

Table 13. Mean and SD of the questionnaires scoring for both groups, before (PRE) and after (POS) Session 2.

VARIABLE	CON (n=5)	EXP (n=7)	*p* value
Anxiety (STAI-S)			
Before	15.40 ±5.31	19.71 ±11.32	0.050
After	14.60 ±3.64	13.14 ±6.84	
POMS total			0.120
Before	4.36±1.01	4.72±1.56	
After	4.29±1.28	3.86 ± 0.75	
Tension (POMS)			0.098
Before	2.13 ±2.66	3.52±2.89	
After	1.80 ± 2.25	1.85±1.82	
Hostility (POMS)			NS
Before	0.33± 0.47	0.61 ±1.63	
After	0.00 ± 0.00	0.95± 0.25	
Fatigue (POMS)			NS
Before	3.40 ±1.09	2.33±2.96	
After	3.13 ±1.99	1.23 ±1.47	
Depression (POMS)			0.108
Before	0.33 ±0.57	1.14 ±2.04	
After	0.80 ±1.26	0.66 ±1.62	
Vigour (POMS)			NS
Before	5.06 ±1.87	5.38±2.13	
After	5.73 ±1.80	5.47±2.21	

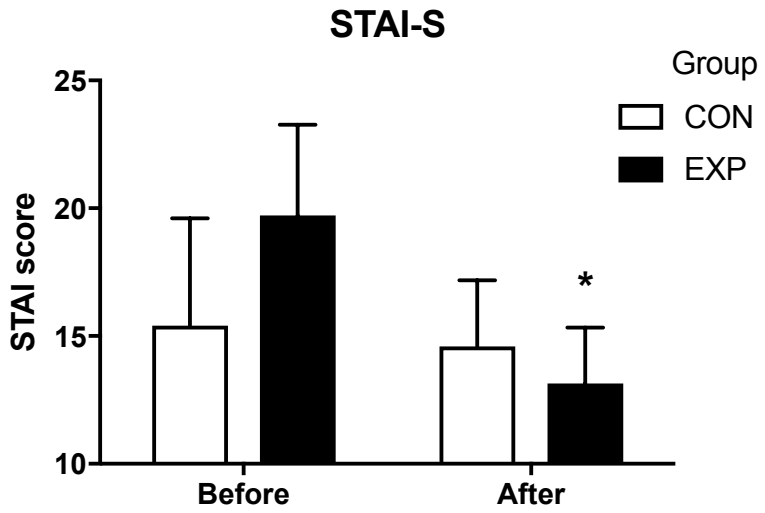


Figure 25. STAI-S scores (Means and SEM) before and after Session 2 for both groups.* EXP: significant decrease, p=0.05 (GLM).

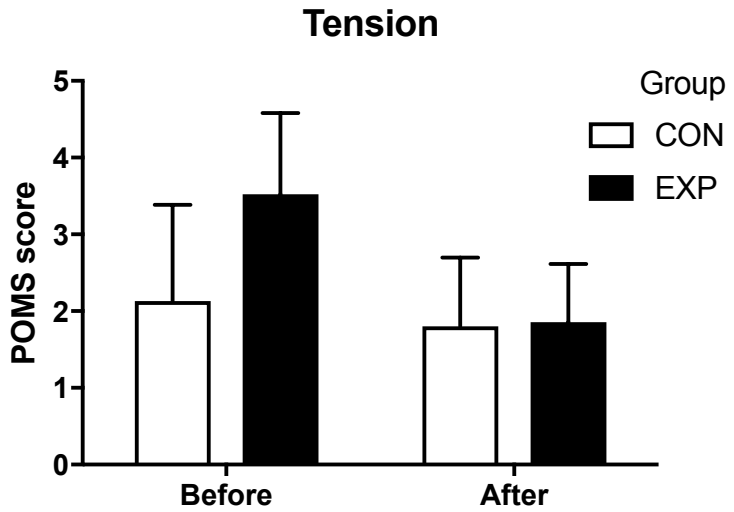


Figure 26. POMS Tension scale scores (Means and SEM) before and after Session 2 for both groups.

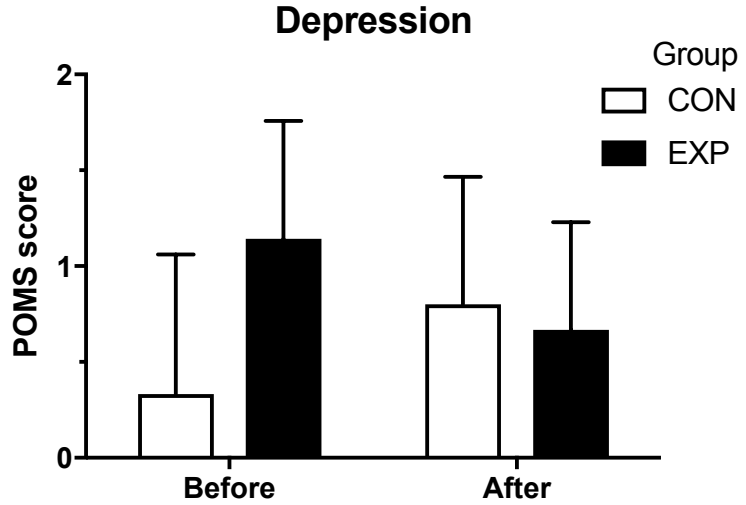


Figure 27. POMS Depression scale scores (Means and SEM) before and after Session 2 for both groups.

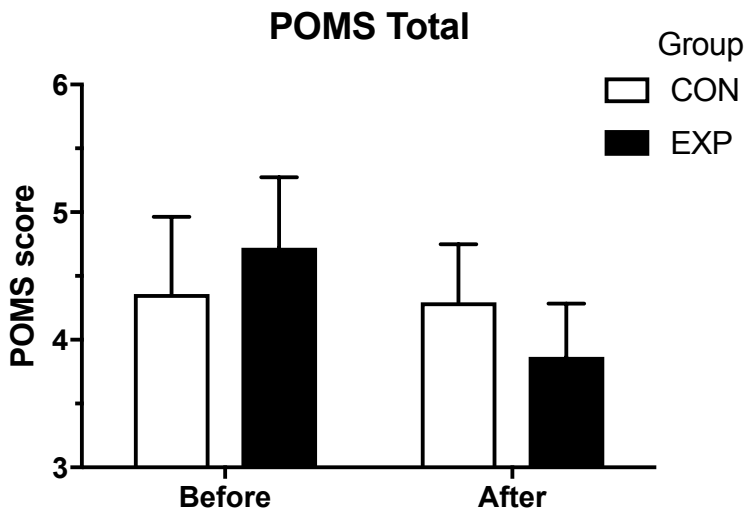


Figure 28. Total POMS scores (Means and SEM) before and after Session 2 for both groups.

5.6 Discussion

This study confirms that Aromatherapy reduces university students' anxiety and increases their concentration in studying, not only in a short term period (45 min) but also in the long term (several weeks). Thus, the inhalation of sweet basil oil through a short session or with an aromatic necklace every day during one month produces emotional and HRV changes on participants.

In our study, we have chosen a very common situation for university students all over the world: 1) a 45 min self-study session at their university school (in a lab situation); 2) one month in their students' life while they prepare a university exam at home. Overall, the results shown in our questionnaire scores indicate a reduction of anxiety and an increase of cardiac variability (tested through HRV analysis). These results could be explained by a balancing effect of AR on the sympathetic/parasympathetic system (ANS).

AR has short time effects because the inhalation of sweet basil oil stimulates the sympathetic activity and increases the secretion of adrenaline. When we compared the aromatherapy group and the control group we could see that the aromatherapy

group showed a greater increase of the SDNN time-domain parameter and LF and LFnu frequency-domain parameters. This incremental effect continued throughout the session. The SDDNN effect can be interpreted by an increase of heart rate variability (Kudo, 2014). In our case, it could mean that AR has a growing relaxing effect which shows a peak at 20-25 min from the beginning of the inhalation. Other studies found changes in SDNN, which can be explained by a stress reduction or a relaxation increase (Luque-Casado, 2013).

At the same time, AR produces a drastic increase of LF and LF% in the same period. The LF parameter can reflect both sympathetic and parasympathetic influences, according to other authors (Reyes del Paso, 2013; Task Force, 1996). In our case, this could be interpreted as an increase of the parasympathetic stimulation, which indicates that, compared to the CON group, the EXP group of students could either experiment less negative emotions (like anxiety or stress) or experiment more positive emotions. In this case, high values in LF could also mean a positive sympathetic stimulation, interpreted as a rise of the arousal level (Luque-Casado, 2013). Therefore, the short term action mechanism of AR can be explained by the balance of the ANS, which is caused by either an increment of the level of arousal (sympathetic activity), or by a reduction of the level of negative emotions (more heart rate variability and less anxiety). The given combination can facilitate the control of the attention level, a critical aspect for university students, whose studying requires high levels of concentration. It is important to remark that this positive effect of AR reaches its peak at 20-25 min from the start of the basil oil inhalation and that this effect continues at least for 45 min (Haze et al., 2002).

The questionnaires results confirm this action mechanism since the EXP group shows a significant decrease of the anxiety level (SATI-S) after Session 2 in comparison with the CON due to the effect of the basil oil inhalation (see Figure 25). This same effect can be observed through the decrease of other negative emotions such as *Tension* scale, *Depression* scale and Total Values for POMS (see Figures 26, 27 and 28).

The main result for the long-time study is that Basil oil inhalation seems to increase the heart rate variability and decrease the anxiety and the negative emotions through the 4 intervention weeks. In Figures 19, 20 and 21 we can observe that RRmean, RMSSD and SDRR show higher POS-PRE increasing through the sessions for EXP. This increasing is not observed for CON. This effect can be interpreted as an increase of cardiac variability (Luque-Casado, 2013). due to the Basil oil inhalation. The maximum HRV increase is produced in Session 3, when EXP students have been inhaling Basil oil for two weeks at home and have had three inhalation sessions in the lab. It could mean that AR has a growing relaxing effect which shows a maximum at 2 weeks from the beginning of the intervention. Other studies found changes in similar HRV parameters, which can be explained by a stress reduction or a relaxation increase (Chang, 2011).

The cognitive results analysed by the questionnaires confirm this explanation. EXP students show significant lower values of negative emotions than CON in Sessions 2, 3 and 4, in POMS for total scoring or for scales like *Tension* and *Fatigue* (see Table 8). However, EXP students show higher values of *Vigour*, the only positive POMS scale, accompanied by low values of fatigue for the last session (the exam day). The EXP group also shows higher values than the CON of physical component (PCS) for SF-12

health survey for Sessions 3 and 4 (Figure 16). Thus, it seems that basil oil improves the perception of better wellness about physical aspect, especially at long term after 2 weeks. It is especially important at Session 4 because it is the exam day for all participants and this wellness questionnaire was used just before starting the exam. After 3-4 weeks of basil oil inhalation, the students feel more active, healthier and less tired. It could be interpreted as an improving effect on the arousal. This state together with a better control over the negative emotion might be appropriate to achieve enough concentration to cope with exams. In fact, students feel better before the exam when they inhaled basil oil the previous weeks (see the results for the Feeling scale in Session 4, Table 7).

After our results we conclude that the use of aromatherapy can be useful to reduce academic anxiety, to improve students' concentration and it might also improve academic performance in a relatively short time (one session) but also at long time (one month). Other investigations support our findings indicating that short term aroma inhalation modulates HRV with the predominance of sympathetic nervous system activity while inducing soothing effects and reducing anxiety. Saeki (2000) demonstrated significant changes in HRV parameters related with sympathovagal balance and associated with relaxation at 5 min after the completion of a foot-bath with lavender oil. We found that the HRV parameters changed around 20-25min after starting the inhalation. In this sense, Kuroda et al. (2005) found that HF power increased more significantly at 21 min than after a 6-min olfactory stimulation of Linalool. In the same line, we found major changes at 20-25 min after starting the inhalation. In one of his studies, Satou (2013) found that an aromatherapy massage could be effective on elderly patients under long-term hospitalization. Meanwhile,

Matsumoto (2013) found that Lavender aromatherapy alleviates premenstrual emotional symptoms with a 3-month intervention. Finally, Igarashi et al. (2014) found that the stimulation with rose and orange oil on the prefrontal cortex activity produces agitation.

The current findings proving that protective aroma effects reduce negative emotions add to increasing evidence supporting that some aromas may protect cognitive functions. The presence of essential oils and their composition determine the specific effect of plants on emotions. For example, for Basil essential oil, this effect can be explained by the combined action of Basil main components. Some of these essential oil components have sedative effects related to YIN (see Figure 4), such as: Esters, Sesquiterpene Hydrocarbons, linalyl acetate and linalool. These components can induce changes on the parasympathetic activity. Some of the essential oil components have activation effects related to YANG, such as Phenol, Aldehyde, Oxides. They can have both an activation and a sedative effect.

Thus, the use of high components concentration could have activation effects. Unlike, the use of low components concentration could have sedative effects. In Figure 14 we can observe the basil main components: linalool (40-55%), methyl chavicol (7%) and phenol 6%. Methyl chavicol is a kind of phenylpropanoid with specific chemical qualities related to Yang but it has a balance effect on the autonomic nervous system. Haze et al. (2002) found that methyl chavicol induced stimulation of sympathetic activity. In the same sense, Schnaubelt (1998) confirms that the phenylpropane ethers, especially estragole (methyl chavicol) stabilize an overactive sympathetic nervous system (sympatholytic effect) and restore a healthy balance between the sympathetic and parasympathetic nervous systems.

Linalool is another component in aroma that could have some activation effects by stimulating the sympathetic activity. Linalool is a natural enantiomer monoterpene commonly found as the major volatile component of essential Oils in several aromatic plant species, which is known to exhibit several biological activities such as CNS depressant, antinociceptive, anxiolytic and anticonvulsant (Peana et al., 2002; Peana et al., 2006; Kamatou & Viljoen, 2008; Batista et al., 2008). Other authors indicate the use of Sweet Basil as a sedative in Spain (Vázquez et al., 1997) and as a sedative and anticonvulsant in Mexico (Tortoriello and Romero, 1992). It also ensures, together with vitamin C, caroten, calcium and phosphorus, skin health and tonicity of the nervous system, consolidating the memory processes. Eugenol is another kind of chemical component in sweet basil oil. It is a phenylpropene, an allyl chain-substituted guaiacol. Eugenol is a member of the phenylpropanoids class of chemical components. It also could be find as a chemical component in clove oil, nutmeg, cinnamon, basil and bay leaf. Also has been used in traditional medical systems to relieve symptoms and cure a variety of diseases (Peana et al., 2002). It has also an adaptive role, with antistress effect, it helps nervous and emotional processes, and it has an immunostimulant effect. Robin et al. (1999) have shown that basic emotions evoked by Eugenol Odor differ according to the Dental experience. One possible explanation of the stimulating effect of the components of Sweet Basil could be that the oil (absorbed via the olfactory system) stimulates the locus coeruleus in the brain into releasing noradrenalin, a neurotransmitter that creates a stimulating/activating effect. The locus coeruleus is also involved in arousal and activation processes. Another possible explanation of its effect could be that sweet basil exerts its effects by an interaction with central structure (e.g. hypothalamic, limbic, thalamic) which controls the level of autonomic

and/or behavioral arousal. Overall, our study agrees with the importance of Sweet Basil as a way to reduce anxiety, raise the level of positive emotion and improve quality of study in university students.

The chemical components of Sweet Basil oil might help the participants' body to obtain a balanced status. Thus, essential oils can have certain "vitality" that helps our body to achieve a healthy state. According to TCM, essential oils can work through a truly holistic therapy, taking into account the mind, the body and the spirit of the person. Essential oils can connect with our body and help it to find the best way to solve its needs. In our case, the final effect can be an improvement of the mental and emotional condition by a combination of reducing the anxiety level before and during an exam and increasing the arousal level of the participants in terms of attentiveness and alertness.

5.7 Conclusions

Our study confirms, with the aid of HRV analysis, that Sweet Basil inhalation can reduce anxiety level and increase study concentration of university students at short time (one-hour session) and at long time (one month). The EXP group reduced academic anxiety level and incremented cardiac variability compared to the CON group. These results could be explained by an autonomic balance on the sympathetic/parasympathetic system through a combined action of the *basil* components: linalool (40-55%), methyl chavicol (7%) and phenol 6%). Some of these components have sedative effects (parasympathetic activity), which belong to YIN in TCM, and other can have activation effects (sympathetic activity), which pertain to YANG in TCM.

6. Pilot Study 2.

6.1 Objective

To conduct a pilot study for determining the procedures and characteristics of aroma methods, based on the meridian system, to improve work performance and reduce workplace stress. This information will be useful to conduct the definitive Study 2 later.

6.2 Sample

The participants were 5 university professors used to working sitting in front of a computer several hours per day (3 male and 2 female; aged from 30 to 53). All participants consented to collaborate voluntarily and provided informed consent before participating in the pilot study. They met the following inclusion criteria: 1) no clinical diagnosis of autonomic dysfunction; 2) no clinical diagnosis of respiratory, cardiovascular, endocrine or systemic disorder; 3) no problems with olfactory function; 4) none of the participants had been clinically diagnosed with diabetes mellitus, hypertension, hyperlipidaemia, asthma, cardiovascular disorders, or any other endocrine or systemic disorders that could affect the autonomic nervous system. All five participants completed a total of 6 sessions in a lab or workplace situation.

6.3 Instruments

For this pilot study we used the FitLab Team system (see part 3.2) to analyse HRV, and the questionnaires STAI-S, POMS, SF12v2 and the Feeling scale (see part 3.1).

6.4 Specific procedure for Pilot Study 2.

We did not inform participants about which fragrance or oil we would use for the study.

We conducted this pilot study through 6 sessions in different weeks and conditions (see Table 14).

Table 14. Characteristics for the 6 sessions conducted in the Pilot Study 2 with university staff.

Session	Participants	Time	Place	Status	Aroma	Use way
Session 1	5	60min	Lab	working	CAB	inhaling
Session 2	5	60min	Office	working	CAB	inhaling
Session 3	5	60min	Lab	working	CL	inhaling
Session 4	5	60min	office	working	CL	inhaling
Session 5	5	90min	office	working	CAB	inhaling
Session 6	5	60min	office	working	CL	inhaling

(CAB: *Citrus aurantium bigarade* (Petitgrain); CL: *Canarium luzonicum* (Elemi)

The participants went to the Lab in 2 group sessions distributed in different weeks. Those sessions were performed in the morning between 9:00AM and 11:00AM in a lab quiet environment. The ambient temperature was controlled between 22 and 25 ºC. The other 4 sessions were performed at the participants' individual work site and they were working in a sitting position in front a computer between 9:00AM and 11:00AM.

In general, the intervention consisted in a 60min aroma inhalation of a specific kind of oil *Elemi* (CAB) and *Petitgrain* (CL) All participants performed a total of 6 sessions in 6 weeks. All participants were asked not to consume any food of beverages containing alcohol or caffeine after the evening of the day preceding the study sessions. They were also asked not to do vigorous physical activity during 24 hours before testing. Participants arrived at lab or at their office and they were asked to rest in a sitting position in front of a table. In general, they followed these steps:

2) Participants were required to fill out the PRE questionnaires (STAI-S, POMS, SF12v2 and the Feeling scale).

3) Participants were asked to wear the cardiac chest band (connected via Bluetooth to an iPad with FitLab software) and sit at rest in front of the table).

4) HRV-Pre (before aroma) was recorded for 5 minutes with the participants at this rest situation: sitting, with their eyes open, placing their hands on their knees, doing no activity, breathing naturally and without speaking.

5) Participants were asked to do a usual work task but in a quiet situation and without speaking to each other. Participants kept this work situation during all the session. Meanwhile, they were inhaling the oil aroma and the HRV was being recorded

6) during the session. The HRV was recorded in a three 5 min periods: 1) During 1, at 10-15min from inhalation starting; During 2, at 20-25 min; and During 3 at 30-35 min.

7) Participants were required to perform a 5 min-HRV-Postest (sitting, with their eyes open, placing the hands on their knees, doing no activity, with natural breathing and without speaking).

8) Participants were asked to fill out the POST questionnaires (STAI-S, POMS and the Feeling scale.

6.5 Results

In this part we will present only the most relevant results of this pilot study for obtaining the key information to design the definitive study (Study 2). Five participants completed the study. The initial participants were university professors of different ages and both sexes and they had to inhale different aroma characteristics in a heterogenic. All participants showed a sufficient compliance with the HRV control variables like no taking medicines, doing low level of physical activity and no drinking coffee before HRV analysis.

Aromatherapy and HRV effects.

In order to check the aromatherapy efficacy to improve work performance and reduce workplace stress, we analyzed the effects of inhaling *Elemi* (CAB) and *Petitgrain* (CL) on HRV through sessions 1,2,5 and 3,4,6 respectively. We performed HRV analysis before, during and after the 6 sessions of the pilot study.

Table 15. HRV Parameters (Mean and SD) for the 5 participants in the 5 session periods.

Aroma	time	RRmean	SDNN	RMSSD	LF/HF	LF%	HF%
	PRE	965.60±169.65	80.26±40.89	62.87±49.01	2.99±2.17	67.44±21.45	32.5±21.45
	Dur 1	956.98±173.145	46.90±31.02	42.03±34.40	2.88±1.64	70.67±11.57	29.39±11.48
CAB	Dur 2	945.98±166.03	88.17±54.85	55.62±44.03	3.56±2.68	66.91±24.98	33.09±24.98
	Dur 3	928.76±174.47	90.44±45.78	57.55±41.98	5.22±3.58	73.68±22.31	26.31±22.31
	POS	915.64±136.01	98.44±53.20	52.55±36.63	6.08±4.83	76.40±18.48	23.60±18.48
	PRE	853.83152.56	81.91± 49.19	58.40 ±21.16	2.90± 3.12	63.33±18.38	36.76±18.39
	Dur 1	876.59± 156.87	66.59±38.72	46.92 ±31.92	4.00± 3.95	61.50±17.86	38.49±17.86
CL	Dur 2	872.93± 152.74	57.72± 29.03	31.53 ±15.31	2.6± 2.26	65.52±14.53	34.37±14.53
	Dur 3	887.94± 169.57	67.57± 31.52	53.59 ±35.23	2.08 ±1.73	55.72±24.78	44.27±24.77
	POS	897.40± 191.98	82.88 ±18.50	68.07± 30.87	1.71± 1.37	57.52±15.09	42.48±15.09

PRE: before inhalation; Dur 1: at 10-15min after the inhalation started; Dur 2: at 20-25 min; Dur 3: at 30-35 min; POS: after inhalation). CL: *Elemi* inhalation (Sessions 3, 4 and 6); CAB: *Petigrain* (Sessions 1, 2 and 5). Dur: during.

Table 15 shows the HRV parameters at baseline (PRE), 10-15min (During 1), 20-25min (During 2) , 30-35min (During 3) and after the inhalation period (POS). The values are means (and SD) for the 5 participants and for the 3 sessions corresponding to each oil (*Elemi* and *Petitgrain*).

A visual inspection of the results shows some differences between both oils in HRV parameters at different intra-session periods.

LF/HF increases at 20-25min, 30-35min and POS for *Petitgrain,* meanwhile it decreases in all these periods for *Elemi* (see Figure 29). Thus, the aroma inhalation seems to have an opposite effect over sympathetic/parasympathetic balance (LF/HF) from 10-15min to the end of the inhalation.

SDRR decreases more at 10-15min when inhaling *Petitgrain* than when inhaling

Elemi, and increases more at 20-25min than when inhaling *Elemi* (see Figure 30). *Petitgrain* maintains higher SDRR values than *Elemi* at 30-35min and POS indicating more heart variability for the first one.

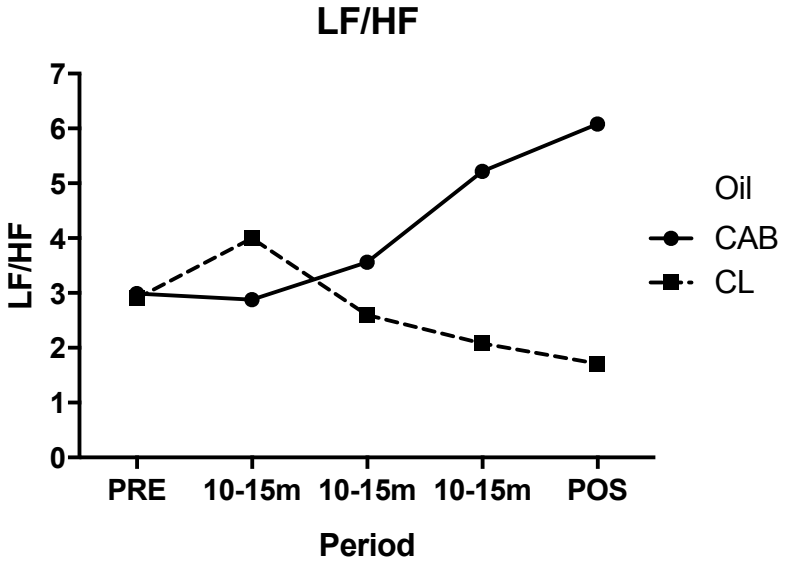


Figure 29. LF/HF mean for the 5 participants in the 5 session periods (PRE-inhalation; at 10-15min from inhalation starting; at 20-25 min; at 30-35 min; and POS, after inhalation), for *Elemi* inhalation (CL; Sessions 3, 4 and 6) and *Petigrain* (CAB, Sessions 1, 2 and 5).

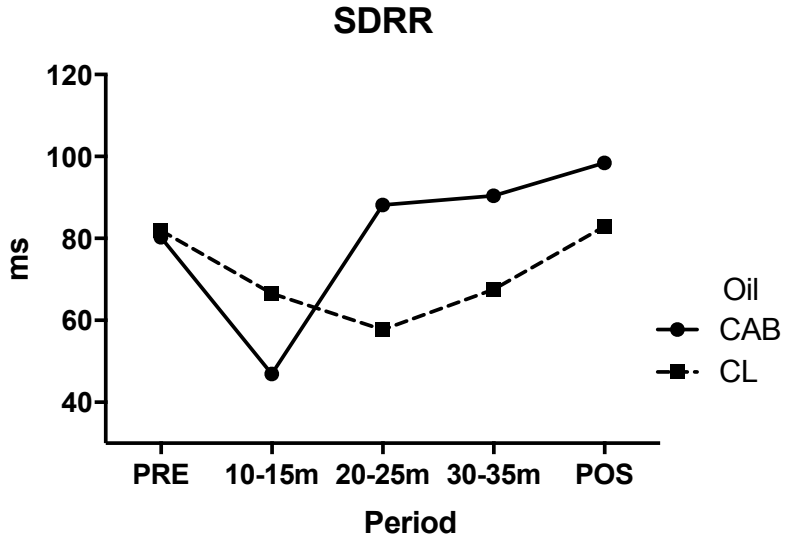


Figure 30. SDRR mean for the 5 participants in the 5 session periods (PRE-inhalation; at 10-15min from inhalation starting; at 20-25 min; at 30-35 min; and POS, after inhalation), for *Elemi* inhalation (CL; Sessions 3, 4 and 6) and *Petigrain* (CAB, Sessions 1, 2 and 5).

6.6 Discussion

The pilot study evaluated the effects that *Petitgrain* and *Elemi* oil have on reducing the level of stress in the workplace and improving work performance. To evaluate these two parameters, we used HRV analysis. We have evaluated these effects on very common situations of administrative university workers: working with computers, writing, reading, etc.

Our data shows differently HRV parameters of *Petitgrain* and *Elemi* after aroma stimulations of 60 minutes. When we compared *Petitgrain* and *Elemi* oil, we found that SDRR showed the same decrease with both *Petitgrain* and *Elemi* oils at 10-15min; LF/HF showed a higher increase with *Petitgrain* than *Elemi a*t20-25min,30-35min and POS; There were no significant differences between the two oil for the ratio RRmean ,RMSSD, HF% and LF%.

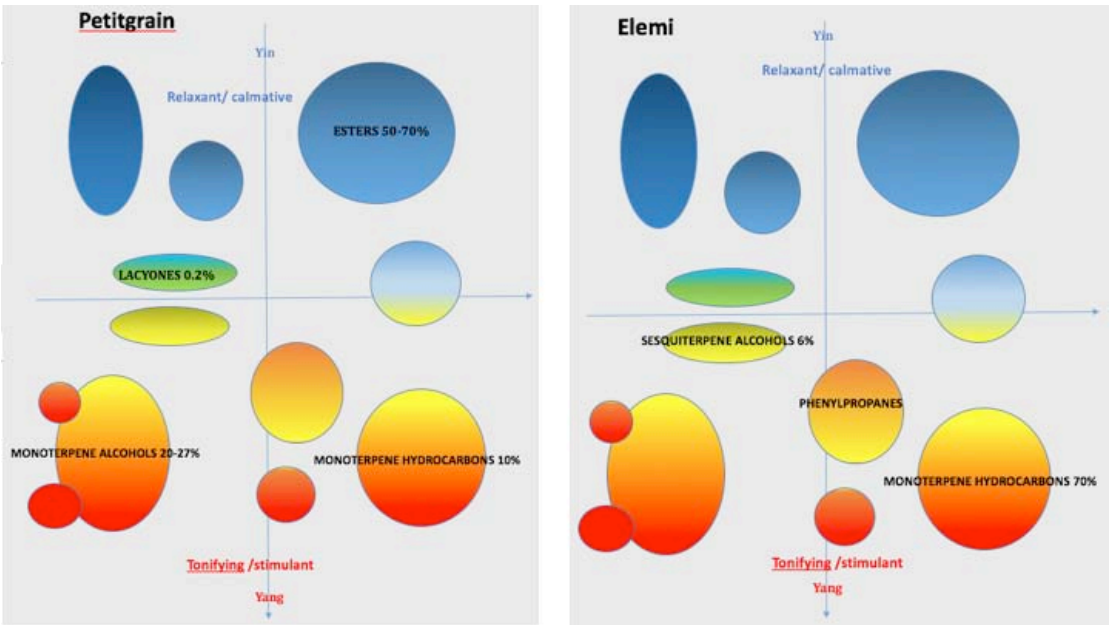
We found that a changed in HRV-parameter related measures following the work stress battery was successfully elicited in participants. When we compared participants before and after inhaling the aroma, we found that *Petitgrain* and *Elemi* had changed the HRV parameter. We also found that after inhaling the aroma of *Petitgrain* and *Elemi* oil for a period of 60 minutes, the stress in the workplace could be reduced and work performance could improve in a relatively short time. The survey shows that sniffing *Petitgrain* and *Elemi* oil can stimulate the parasympathetic nervous system activity in a very short time.

The data shows a deeper Increase on HF/LF after inhaling *Petitgrain oil,* That means petitgrain oil could be trend to increase their heart variability.

This result could be explained by the combined action of *Petitgrain* and *Elemi* oil main chemical composition.

Some studies confirm that the components that have sedative effects (wich belong to *Yin*. such as Esters, Sesquiterpene Hydrocarbons, sesquiterpene alcohols and lactones etc. could induce changes on the parasympathetic activity. Other components could have some activation effects by stimulating the sympathetic activity.could be explain to *Yang*(Haze, Sakai and Gozu,2002).

The predominant constituents of *Petitgraint* are linalyl acetate, linalool and myrcene. Some of these components, such as linalyl acetate and linalool, have a sedative effect, which could induce changes on the parasympathetic activity (Kuroda K, Inoue N, Ito Y, et al. 2005). Myrcene is another component in aroma plants that could have some activation effects by stimulating the sympathetic activity. Myrcene, a kind of monoterpene (1.3–2.12%), could possibly be related to the release of norepinephrine, a neurotransmitter linked to the sympathetic activity stimulation (Franchomme,Jollois,penoel,2001). The predominant constituents of *Elemi* are phellandrenes, elemenes, elemol (Franchomme, Jollois,penoel,2001) (See figure 31).



Figuer 31. Comparison main chemical composition of CAB and CL

In the figure 31 we can see that the Elemi components, which could have some activation effects by stimulating the sympathetic activity, belong to *Yang*; while the *Petitgrain* main components can be found both in Yin and Yang (Franchomme, Jollois, penoel,2001).

The olfactory neuroanatomy is intertwined via extensive reciprocal axonal connections, frontal cortex and other parts of the limbic system. The odour molecules of essential oils are transmitted to the brain by olfactory sensory neurons in the nasal cavity. Thus, they stimulate smell–memory work and influence memory, thoughts, and emotions (Kruemark ,Novak,,Gitelman elte,2013).

The limited of this study is the living habits of participants affected the rule of experimental requirements, such as drinking coffee in the morning. Despite repeated reminders, some participants did not perform well.

We also found that all the oils are useful for participants to improve a negative emotion. We may conduct another study to research on other oils in the future.

6.7 Conclusions

We have analysed *Petitgrain* and *Elemi* HRV parameter and we found that in *Petitgrain* oil the HRV parameter HF/LF was higher than in *Elemi* oil. Recently, some studies have shown that *Petitgrain* has sedative and anxiolytic-like effects, such as the work of Lehner et al. who found that *Petitgrain* reduced the anxiety level of patients, specifically women, when this kind of oil was scattered in the lobby of a dental office. Another study showed that *Petitgrain* (administered orally and derived from the petals and stamens of *petitgrain* oil) reduced the preoperative anxiety of patients scheduled for elective minor surgery.

In the Figure 31, we can see how *Elemi* components are closer to *Yang* and it could have some activation effects by stimulating the sympathetic activity; while *Petitgrain* main components belong both to *Yin* and *Yang*.

The study was focused on determining whether *Petitgrain* and *Elemi* can be used to improve work performance and reduce workplace stress. The results of pilot study 2 showed that *Petitgrain* works better to balance the sympathetic/parasympathetic system and improve negative emotion .

7. STUDY 2

7.1 Objective

The purpose of this study is to analyse the efficacy of aromatherapy to improve work performance and to reduce workplace stress. Specifically, we will check the effects of smelling *patitgrain* on a simulated computer work session to improve the performance time when typing on the keyboard. At the same time, we will analyse the possible effects over HRV and mood/anxiety state questionnaires to explain the results in terms of sympathetic/parasympathetic balance, stress reduction and changes of arousal or attention level.

7.2 Sample

42 administrative university workers composed the initial sample (age: 42.21 years, SD=7.12; gender: 10 men and 32 women). All participants met the following inclusion criteria: (1) age between 26 and 55 years; (2) no clinical diagnosis of autonomic dysfunction; (3) no clinical diagnosis of respiratory, cardiovascular, endocrine or systemic disorder (4) no problems with olfactory function. All participants consented to collaborate voluntarily and provided informed consent before participating in the study. The ethics committee of the University approved the study in advance.

A randomized controlled trial was used. The participants were recruited from a university administrative staff through an institutional email advertisement. All of them were used to working sitting and typing in front of a computer for most of the working day. The participants were randomly assigned to the aromatherapy group (AG) and the control group (CG), and they were invited on different days to participate in

specific sessions for each group. They were not aware of which group they were assigned. Explanations were provided regarding the nature and purpose of the study: to investigate coping effects of plant fragrance on workplace stress. All participants were asked not to consume any food or beverage containing alcohol or caffeine after the evening of the day preceding the experiment. They were also asked not to do vigorous physical activity during 24 hours before testing.

7.3 Instruments:

For this study we used the FitLab Team system (see part 3.2) to analyse HRV and the questionnaires STAI-State, POMS and the Feeling Scale (see part 3.1). The participants inhaled the essential oils through four diffusers that allowed a proper smelling throughout the room. We used two kinds of oils, one for each group: 1) AG inhaledil *Petitgrain* (*Citrus aurantium (Fe);* abbreviation: CA; Italian, Production batch: IF745, Neroly Company Limited, Hong Kong). 100% pure CA was used and diluted to 2%. The predominant constituents were linalyl acetate (45-55％), linalool (20-27％) and myrcene (1.3-12.12%). 2) CG inhaled *Sweet almond* oil (*Prunus amygdalus var. dulcis*; abbreviation: PAV; American, Production batch: YA637, Neroly Company Limited, Hong Kong). The predominant constituents were palmitoleic acid (60-68%), linoleic acid (17-30%) and palmitic acid (6-8%). PAV is a vegetable oil and has a neutral effect for smelling.

7.4 Procedure

We randomly distributed the participants into 6 sessions (7 subjects each) in different days, 3 for CG and 3 for CG. All sessions were performed in a university computer classroom between 10:00 AM and 11:30 AM and each participant came only

once. When the participants arrived at the computer classroom, they were asked to wear the cardiac chest band (Bluetooth connected) and to rest in a sitting position in front of a computer, like in their workplace. Firstly, participants were required to fill out the questionnaires STAI-State, POMS and the Feeling scale. Secondly, HRV-Pre was tested for 5 minutes with the participants at rest (sitting, with their eyes open, placing their hands on their knees, doing no activity, breathing naturally and without speaking). Third, at the end of HRV-Pretest, the oil diffusers were launched and worked throughout the session with CA for AG sessions and PD for CG sessions (Figure 1, A). At the same time, a researcher explained for about 15 minutes how to complete a neutral computer task in a specific website. (see Figure 32)

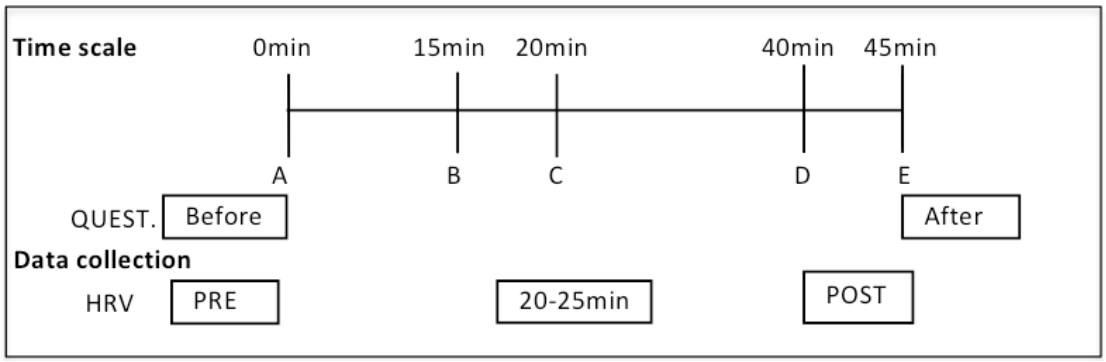


Figure. 32. Experimental procedures and data collection. Before: STAI and POMS questionnaires. PRE: 5min HRV Pre-Test. A: Inhalation beginning. B: Computer task beginning. C: 5min HRV "20-25min"-Test. D: Computer task finishing (individual "Performance Time"). POST: 5min HRV Post-Test (step D to E). After: STAI and POMS.

This task was the same for all participants and consisted on completing 10 standard forms. All participants began individually to complete this task typing on their keyboard until they finished it (step B to D in Figure 32). The single times (B to D) were different for all participants and were recorded in the website as "Performance Time". A period of 5 min-HRV (Figure 32, C) was recorded at 20 to 25 minutes from the beginning of the inhalation period (A). Forty minutes after the inhalation beginning (A),

all participants had finished their website task and their HRV was recorded during a period of 5 minutes (POST; step D to E in Figure 1), in the same conditions as the HRV-Pretest. Finally, participants were asked to fill out the POST questionnaires (STAI-State, POMS and the Feeling scale).

7.5 Results

Among the 42 initial participants, 5 did not complete the study because they showed

Too many artefacts in the HRV recordings. For the AG, 20 out of 21 participants completed the study, and for CG the number of participants was 17 out of 21. There were no statistically significant differences between Aromatherapy (AG) and CG in age (AG: 43.0±6.4 years old; CG: 43.2±6.8 years old) or in gender (AG: 20% of male and 80% differences between the two groups were found by HRV control variables like taking medicines, doing physical activity and drinking coffee.

The ONE WAY analysis of variance shows that AG performed faster than CG the 10 standard forms typing them with the keyboard in the website: the *performance time* for AG was 17.35±3.13 min, and for CG was 19.63±3.87min (p=0.05) (see Table 16).

Table 16. Gender (%), Age and *Performance Time* (M±SD) for the two groups.

Variable	**Control** (n=17)	**Aroma** (n=20)	***p* value**
Age (years)	43.21±6.80	43.00±6.44	NS
Gender			
male	31.6 %	20%	NS
female	68.4 %	80%	
Performance Time (min)	19.63±3.87	17.35±3.13	0.05

Male: CG: 31.6% of men and 68.4% of female . No significant

Table 17. HRV parameters (M±SD) in the three HRV session recordings (PRE, 20-25min, and POST) for the two groups.

HRV parameter	**Control** (n=17)	**Aroma** (n=20)	**Total** (n=37)	***p* value**
RRmean (ms)				
PRE	854.64±118.91	870.10±92.10	863.00±104.03	<.001[a]
20-25min	865.35±114.52	884.85±107.01	875.89±109.40	
POST	896.88±112.69	907.35±103.02	902.54±106.17	
HR (bpm)				
PRE	71.50±9.73	69.70±7.65	70.58±8.59	<.001[b]
20-25min	69.41±7.99	67.83±7.65	68.60±7.76	
POST	67.77±8.09	66.89±7.27	67.32±7.59	
SDNN (ms)				
PRE	45.64±14.19	54.10±21.76	50.21 ±18.91	0.05*
20-25min	50.47±16.88	48.45±16.84	49.37±16.65	
POST	57.76±22.40	56.90±23.96	57.29±22.94	
RMSSD (ms)				
PRE	26.58±8.50	33.30±17.90	30.21±14.59	0.02*
20-25min	31.23±8.37	32.65±21.07	32.00±16.31	
POST	32.94±11.96	32.20±13.67	32.54±12.74	
LF/HF (ratio)				
PRE	4.17±4.21	4.36±5.48	4.27±4.87	NS
20-25min	5.53±6.04	3.04±2.49	4.18±4.59	
POST	5.73±7.12	3.45±1.88	4.50±5.07	
LF (ms^2)				
PRE	1013.05±1036.89	1222.05±1426.21	1126.02±1250.01	0.05*
20-25min	990.94±731.01	739.15±605.55	854.83±668.74	
POST	1514.35±1695.93	1025.20±737.48	1249.94±275.31	
HF (ms^2)				
PRE	258.52±156.12	498.30±560.25	388.13±437.22	0.02*
20-25min	266.58±165.24	576.45±1066.77	434.08±798.28	
POST	346,29±218.50	393.15±367.22	371.62±304.88	
VLF (ms^2)				
PRE	779.00±652.38	1258.25±1052.01	1038.05±12.08	0.058[c]
20-25min	1167.76±986.78	932.75±663.68	1040.73±824.22	
POST	1815.76±1949.89	1957.55±2182.60	1892.40±2051.62	
LFnu (%)				
PRE	71.11±15.42	69.50±17.92	70.24±16.61	0.08[c]
20-25min	74.64±14.81	66.20±18.26	70.08±17.08	
POST	70.94±20.81	73.10±11.88	72.10±16.37	
HFnu (%)				
PRE	28.22±15.42	30.50±17.92	29.75±16.61	0.08[c]
20-25min	25.35±14.81	33.80±18.26	29.91±17.08	

POST	29.05±20.81	26.90±11.85	27.89±16.37

*Significant differences between the two groups; [a]Total differences for all sample: POST>PRE; [b]Total differences for all sample: PRE>POST; [c]Trend to significant differences between the two groups; NS: no significance.

Table 17 shows the differences in the HRV parameters between the two groups at baseline (PRE), at the 20-25 min from the starting of smelling period and after the inhalation period (POS).

An analysis of variance according to a general linear model (GLM) shows differences between the two groups in terms of evolution throughout of the three stages of the session (PRE, 20-25min, and POS) for the following HRV spectral parameters: LF, such that AG shows a larger LF decrease between PRE and 20-25min than CG, and CG shows a larger LF increase between 20-25min and POS than AG ($p=0.05$; see Figure 33).

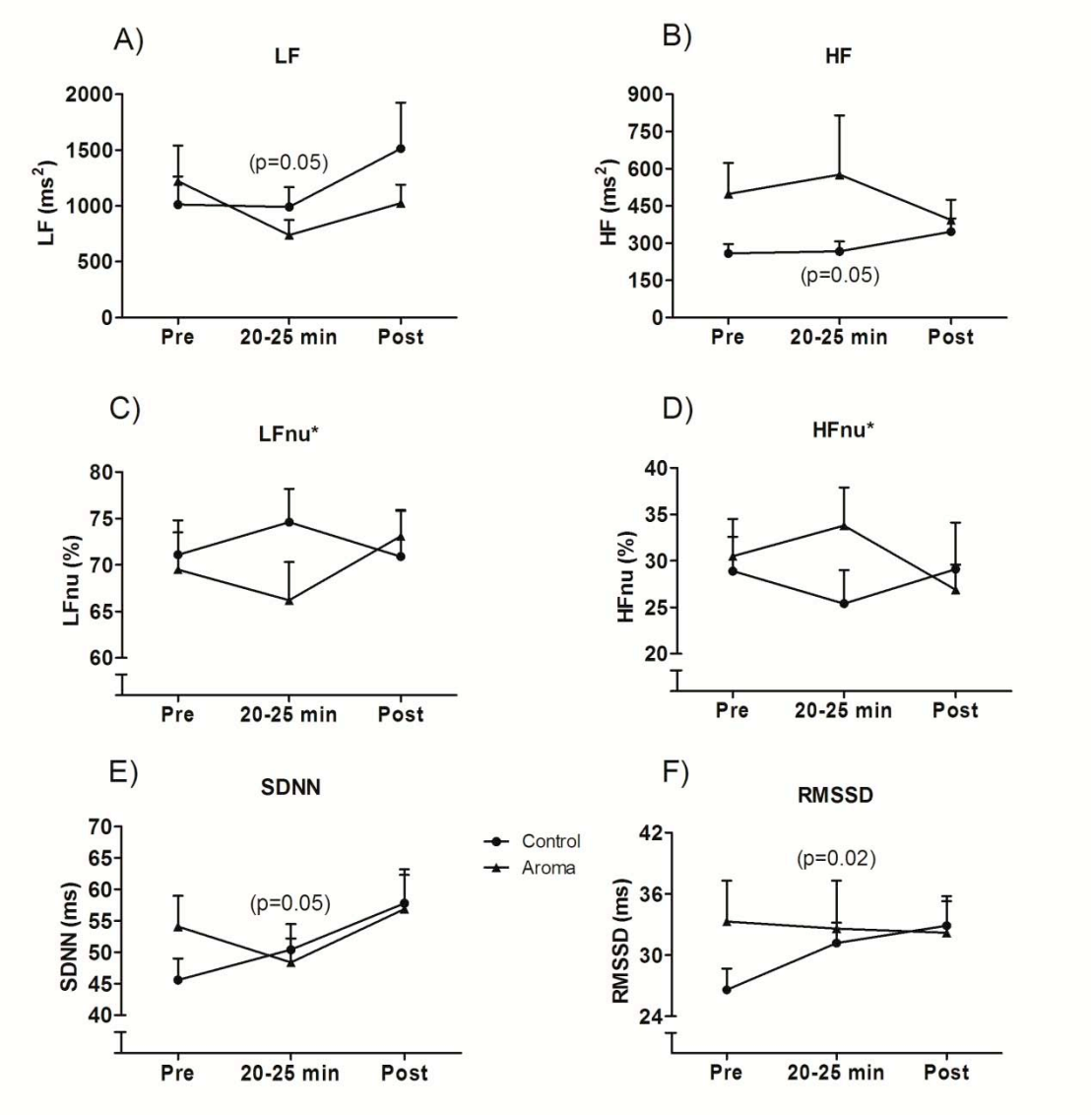


Figure. 33. Comparison of HRV parameters during the smelling period between the two groups at baseline (PRE), at the 20-25 min period and after the inhalation period (POS): A) low frequency band (LF); B) high frequency band (HF); C) LF values expressed in normalized units (LFnu; *p=0.08); D) HF values expressed in normalized units (HFnu; *p=0.08); E) standard deviation of all RR intervals (SDNN); F) root mean square of differences of successive RR intervals (RMSSD).

A); HF, such that AG shows a larger HF decrease between 20-25min and POS than CG, meanwhile CG shows an HF increase in this stage (p=0.02; see Figure 2, B). Figure 2 (parts C and D) shows the differences between groups with trend to significance for HFnu (p=0.08) and LFnu (p=0.08). VLF parameter also shows a trend towards statistical significance (p=0.058). GLM analysis shows differences between the two groups for the time domain HRV parameters: SDNN (p=0.05; Figure 2, E); and RMSSD (p=0.019; Figure

2, F). There were no significant differences between the two groups for the ratio LF/HF, RR mean and heart rate (HR) mean.

There are no differences in the questionnaires scorings between the two groups in terms of evolution from before (PRE) to after (POST) the session. But there were significant differences for all participants, not depending on the group, between PRE and POST for STAI ($p<0.001$), Tension ($p<0.001$) and Vigour ($p<0.015$) scales. In all cases the participants showed a decrease from PRE to POST.

7.6 Discussion

Our study agrees with the importance of aromatherapy as a way to improve quality of life and performance in the workplace. We found that smelling CA in a simulated computer work session improves the performance time when typing with the keyboard and produces some changes on the heart rate variability (HRV). Thus, the Aromatherapy Group (AG, smelling CA) ends more than 2 minutes before than the Control Group (CG, smelling PD), when doing the same task, which consists on completing specific web forms. We aim to explain this result as a combination of reducing the stress level and increasing the attention level of the participants, with the aid of the HRV analysis.

In our study we chose a very common workplace task in the developed countries. Typing on the keyboard in front of a computer during a full workday is a usual task for a lot of jobs like university staff, bank staff or administrative staff of any company. Our participants were administrative university staff and we chose a non-specialized computer task. It was to complete a limited number of questionnaires and self-reports about the own lifestyle. Thus, there were no right or wrong answers and all

participants knew the answers. The best performance consisted on thinking the answers quickly and typing on the keyboard in a fast way. All items had to be completed before ending and clicking the last option ("Save"). We promised an individualized report to participants about their lifestyle and all of them completed the items with coherent answers. We randomly distributed the participants in two groups, so they were balanced in terms of mental and typing agility. In these conditions, we have to attribute the performance improvement of AG group (more than 2 min better than CG) showed in our results to the aromatherapy effects (smelling CT).

The job stress-related problem is currently increasing and potentially affecting employee performance and personal health (Embriaco N, Papazian L, Kentish-Barnes N 2007). We can find studies about relieving mental load in work places through strategies such as analysing the exercise effects on autonomic balance (Moreno , Ramos, and Capdevila,2015) or about the effects of aromatherapy in alleviating work-related stress(Liu ,Cheng ,Wang *et al* ,2015) A growing number of studies describe the efficacy of aromatherapy treatment in this field (Liu , Lin , Chang 2013). However, few studies examine the effects of aromatherapy directly on work performance through the autonomic balance. In our study, we have contributed to this using HRV analysis to explain the performance improvement in Aromatherapy Group by a stress reduction due to the aromatherapy effect.

This stress reduction could be explained by a specific effect on the autonomic nervous system. For this purpose, in our study we analysed HRV in three 5-min-periods, first before smelling, second during smelling after 20 minutes from the beginning, and third after the smelling period. Our HRV main findings reveal significant differences between both groups in HF and LF related parameters. These indices have

been used to reflect primarily sympathetic and parasympathetic influences (Matsumoto, Asakura, Hayashi,2013). The larger LF decrease between PRE and 20-25min in AG could be explained by a specific effect of smelling CA reducing the sympathetic activity during the task performance. Otherwise, the sympathetic effect could be effective during the last session period, where CG shows a larger LF than AG. In this last period (between 20-25min and POST) the parasympathetic activity (showed by HF) is different in both groups. We can see this same trend for the standardized parameters. Thus, the most important aromatherapy effect is the increased parasympathetic activity (HF and HFnu increases) for AG group between PRE and 20-25 period, combined with a decline of the sympathetic activity (LF and LFnu decreases) for this group in the same period. These changes could produce a sympathetic-parasympathetic balance of the ANS improving the attention control and facilitating the performance. In addition, the significant decrease of SDNN parameter in AG during the task (PRE and 20-25min) means a minor cardiac variability than CG, and could be interpreted as an increasing of arousal. This higher arousal could permit AG to pay more attention to the task improving the performance. Thus, smelling CT could improve the mental and emotional condition in terms of attentiveness and alertness.

We also analysed the cognitive levels of anxiety and mood state as stress symptoms in our study. But we did not find differences between the two groups. We found significant differences for all participants for anxiety scores (STAI) and psychological tension (POMS). They showed less anxiety values after the session, but these global results were expected because they had just performed a theoretical stressful task. Maybe we would need more than one aromatherapy session to obtain

more cognitive anxiety reduction in AG. But, what is the mechanism of action underlying the performance improvement effect of smelling CA? Aromatherapy could drive autonomic nervous activity towards a balanced state (Chang and Shen,2011). This balance could be explained by the combined action of CA main components. On the one hand, some of these components have sedative effects, such as linalyl acetate and linalool, which could induce changes on the parasympathetic activity (Igarashi 2013) In this sense, Kuroda et al. found that HF power increased more significantly at 21 min after a 6 min olfactory stimulation of linalool (Kuroda, Inoue , Ito , *et al*.2005) In the same line, we found changes at 20-25min after starting the smelling period. Thus, CA seems to have an increasing effect on parasympathetic activity in AG (HF and HFnu), which was clearly higher at the end of the session (POST). Meanwhile, CG decreased HF and HFnu values in POST period. On the other hand, myrcene is another component in CA that could have some activation effects by stimulating the sympathetic activity (Herz RS.2005). Myrcene, a kind of monoterpene (1.3-2.12%), could possibly be related to the release of norepinephrine, a neurotransmitter linked with the sympathetic activity stimulation (Chang and Shen,2011). This could help to explain our results as an increasing of the arousal levels in AG. Similar results were found with pepper oil smelling, which induced an increase in adrenaline concentration, while inhalation of rose oil decreased the adrenaline level. The same stimulating effects of essential oils on sympathetic and parasympathetic activity have been reported, e.g. estragon oil or grapefruit oil. These essential oils, as pepper oil, consist of some components, such as limonene, pinene or methyl chavicol, which possibly mediate the stimulating effect on sympathetic activity. Furthermore, alcohols such citronellol, geraniol, nerol and patchouli alcohol were detected in rose oil and

patchouli oil, which induced inhibition of parasympathetic activity (Haze, Sakai, Gozu 2002).

The olfactory system interactions could be another mechanism of action to explain the effects of CA through an interaction with central nervous system structures (e.g. hypothalamic, limbic, thalamic), which control the level of autonomic and behavioural arousal (Bowers 2006). The olfactory neuroanatomy is intertwined, via extensive reciprocal axonal connections, with primary emotion areas including the amygdala, hippocampus, and orbitofrontal cortex and other parts of the limbic system. The odour molecules of essential oils are transmitted to the brain by olfactory sensory neurons in the nasal cavity; thus, they stimulate smell–memory work and influence memory, thoughts, and emotions (Stea, Berardi , Pasquale, 2014). Further studies are needed to evaluate the olfactory system interactions and another possible action mechanisms underlying the workplace stress and performance improvement effect of aromatherapy.

7.7 Conclusions

Our study confirms that aromatherapy (inhaling petit grain essential oil) can improve the performance in the workplace. The most important aromatherapy effect is the increased parasympathetic activity for AG group between PRE and 20-25 period, combined with a decline of the sympathetic activity for this group in the same period. These changes could produce a sympathetic-parasympathetic balance of the ANS improving the attention control and facilitating the performance. This balance could be explained by a combined action of the petit grain main components (like linalyl acetate, linalool or myrcene). On the one hand, some of these components have

sedative effects, which could induce changes on the parasympathetic activity. On the other hand, other components could have some activation effects by stimulating the sympathetic activity. The final effect could be an improvement of the mental and emotional condition by a combination of reducing the stress level and increasing the arousal level of the participants in terms of attentiveness and alertness.

8. General Conclusions

1. This doctoral thesis confirms that aromatherapy (inhaling essential oils) can improve performance in the workplace, can help to manage negative emotions and can increment attention level in academic and workplace situations.

2. Our results confirm that inhalation of essential oils produces this effect by balancing the sympathetic/parasympathetic system of the Autonomic Nervous System. HRV analysis is a good marker of this effect.

3. Inhalation of *Petitgrain* essential oil reduces stress and improves performance in the workplace (Study 2). AR improves the performance time when typing with the keyboard in a simulated computer work session and produces some changes improving HRV.

4. Inhalation of *Basil* essential oil reduces academic anxiety and improves study concentration of university students (Study 1). AR in a short session or with an aromatic necklace every day during one month produces emotional and HRV changes on participants.

5. Inhalation of essential oils has its most acute effects at 20-25 min after starting inhaling, both with *Petitgrain* and *Basil* oils. It means it will be enough to program 30-min sessions with a short-time effect to reduce negative emotions and improve performance in academic and workplace situations.

6. AR has short-term efficacy (1 hour) to reduce negative emotions and improve performance in academic and workplace situations, and long-term efficacy (1 month) to reduce negative emotions in academic situations.

7. This ANS balance can be explained by a combined action of the *Petitgrain* and *Basil* main components. Some of them have sedative effects, which can induce changes on the parasympathetic activity, and other components have activation effects, which stimulate the sympathetic activity. The final effect can be an improvement of the mental and emotional condition by a combination of reducing stress level and increasing arousal level of the participants in terms of attentiveness and alertness.

8. We can also explain our results according to TCM. Essential oils can work through a truly holistic therapy, taking into account the mind, the body and the spirit of the person. Essential oils can connect with our body and help it to find the best way to solve its needs. The relaxing effects (parasympathetic activity) can be related to YIN and the activation effects (sympathetic activity) can be related to YANG.

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10. Annexes.

Annex 1. Original questionnaires used in the studies.

Laboratori de Psicologia de l'Esport, UAB

Aromatherapy Study

Researcher: Huang Lin

Data: ____/____/____ Hora:_____
DNI: _____ (Si no recordes el DNI, identifica't amb el **NIU: _____**) **Sexe: ○ Dona ○ Home Data de Naixement: ____/____/19__**
Edat: _____ Pes: _____Kg. Alçada: _____cm.
Instruccions Si us plau, llegeix acuradament les diferents instruccions de cada qüestionari i contesta sincerament totes les preguntes, sense deixar-ne cap en blanc.

Control de la HRV	
INSTRUCCIONES: **A continuación, te haremos algunas preguntas sobre aspectos que pueden influir en los resultados del estudio.**	
1. ¿Has tomado alguna medicación durante las últimas 24 horas?	☐ Sí ☐ No ¿Cuál/es?_____ _____ _____ ¿A qué hora? _____
2. ¿Has hecho actividad física durante las últimas 20 horas?	☐ Sí ☐ No ¿Qué tipo? _____ ¿A qué hora? _____ ¿Durante cuánto tiempo?_____ ¿Cuántas horas han pasado desde que acabaste la actividad?_____
3. ¿Has tomado cafeína hoy?	☐ Sí ☐ No ¿Cuántas horas hace? _____
4. ¿Has fumado hoy?	☐ Sí ☐ No ¿Cuántas horas hace del último cigarro? _____
5. ¿Has tomado alguna bebida alcohólica durante las últimas 10 horas?	☐ Sí ☐ No
6. ¿Has realizado alguna comida copiosa en las últimas tres horas?	☐ Sí ☐ No
7. ¿Has comido alguna cosa en la última hora?	☐ Sí ☐ No
8. ¿Cuántas horas has dormido esta noche? _____ _____ ¿Crees que el tu sueño ha sido de calidad?	☐ Sí ☐ No

Su Salud y Bienestar

Las preguntas que siguen se refieren a lo que usted piensa sobre su salud. Sus respuestas permitirán saber cómo se encuentra usted y hasta qué punto es capaz de hacer sus actividades habituales.
***Gracias por contestar a estas preguntas*.**

Para cada una de las siguientes preguntas, por favor marque con una ☒ la casilla que mejor corresponda a su respuesta.

1. En general, usted diría que su salud es:

Excelente	Muy buena	Buena	Regular	Mala
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. Las siguientes preguntas se refieren a actividades o cosas que usted podría hacer en un día normal. <u>Su salud actual, ¿le limita para</u> hacer esas actividades o cosas? Si es así, ¿cuánto?

	Sí, me limita mucho	Sí, me limita un poco	No, no me limita nada
a <u>Esfuerzos moderados</u>, como mover una mesa, pasar la aspiradora, jugar a los bolos o caminar más de 1 hora.	☐ 1	☐ 2	☐ 3
b Subir <u>varios</u> pisos por la escalera.	☐ 1	☐ 2	☐ 3

3. Durante <u>las 4 últimas semanas</u>, ¿con qué frecuencia ha tenido alguno de los siguientes problemas en su trabajo o en sus actividades cotidianas, <u>a causa de su salud física</u>?

	Siempre	Casi siempre	Algunas veces	Sólo alguna vez	Nunca
a ¿<u>Hizo menos</u> de lo que hubiera querido hacer?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b ¿Tuvo que <u>dejar de hacer algunas tareas</u> en su trabajo o en sus actividades cotidianas?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. Durante <u>las 4 últimas semanas</u>, ¿con qué frecuencia ha tenido alguno de los siguientes problemas en su trabajo o en sus actividades cotidianas, a causa de <u>algún problema emocional</u> (como estar triste, deprimido, o nervioso)?

	Siempre	Casi siempre	Algunas veces	Sólo alguna vez	Nunca
a ¿<u>Hizo menos</u> de lo que hubiera querido hacer <u>por algún problema emocional</u>?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b ¿Hizo su trabajo o sus actividades cotidianas menos <u>cuidadosamente</u> que de costumbre, <u>por algún problema emocional</u>?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. Durante las <u>4 últimas semanas</u>, ¿hasta qué punto <u>el dolor</u> le ha dificultado su trabajo habitual (incluido el trabajo fuera de casa y las tareas domésticas)?

Nada	Un poco	Regular	Bastante	Mucho
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. Las preguntas que siguen se refieren a cómo se ha sentido y cómo le han ido las cosas durante las 4 últimas semanas. En cada pregunta responda lo que se parezca más a cómo se ha sentido usted. Durante las últimas 4 semanas ¿con qué frecuencia...

	Siempre	Casi siempre	Algunas veces	Sólo alguna vez	Nunca
Se sintió calmado y tranquilo?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Tuvo mucha energía?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
Se sintió desanimado y deprimido?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. Durante las 4 últimas semanas, ¿con qué frecuencia la salud física o los problemas emocionales le han dificultado sus actividades sociales (como visitar a los amigos o familiares)?

Siempre	Casi siempre	Algunas veces	Sólo alguna vez	Nunca
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

STAI A-E

(PRE and POS)

INSTRUCCIONES

A continuación encontrará unas frases que se utilizan corrientemente para describirse uno a sí mismo.

Lea cada frase y señale la puntuación de 0 a 3 que indique mejor **CÓMO SE SIENTE USTED AHORA MISMO,** en este momento**.** No hay respuestas buenas ni malas. No emplee demasiado tiempo en cada frase y conteste señalando la respuesta que mejor describa su situación presente.

0	NADA
1	ALGO
2	BASTANTE
3	MUCHO

	Nada	Algo	Bastante	Mucho
1. Me siento calmado.	0	1	2	3
2. Me siento seguro.	0	1	2	3
3. Estoy tenso.	0	1	2	3
4. Estoy contrariado.	0	1	2	3
5. Me siento cómodo (estoy a gusto).	0	1	2	3
6. Me siento alterado.	0	1	2	3
7. Estoy preocupado por posibles desgracias futuras.	0	1	2	3
8. Me siento descansado.	0	1	2	3
9. Me siento angustiado.	0	1	2	3
10. Me siento confortable.	0	1	2	3
11. Tengo confianza en mí mismo.	0	1	2	3
12. Me siento nervioso.	0	1	2	3
13. Estoy desasosegado.	0	1	2	3
14. Me siento muy "atado" (como oprimido)	0	1	2	3
15. Estoy relajado.	0	1	2	3
16. Me siento satisfecho.	0	1	2	3
17. Estoy preocupado	0	1	2	3
18. Me siento aturdido y sobreexcitado.	0	1	2	3
19. Me siento alegre.	0	1	2	3
20. En este momento me siento bien.	0	1	2	3

Estado de Ánimo. *(PRE and POS)*

INSTRUCCIONES:
A continuación aparece una lista de palabras utilizadas por la gente para describir sus sensaciones. Lee cada palabra o frase y marca el número apropiado que aparece a la derecha para indicar ***cómo te sientes en este momento***.

En este momento me siento:	**Nada**			**Algo**				**Bastante**		**Mucho**
1. Tenso/a	0	1	2	3	4	5	6	7	8	9	10
2. Enfadado/a	0	1	2	3	4	5	6	7	8	9	10
3. Agotado/a	0	1	2	3	4	5	6	7	8	9	10
4. Infeliz	0	1	2	3	4	5	6	7	8	9	10
5. Activo/a	0	1	2	3	4	5	6	7	8	9	10
6. Enérgico/a	0	1	2	3	4	5	6	7	8	9	10
7. Fatigado/a	0	1	2	3	4	5	6	7	8	9	10
8. Desanimado/a	0	1	2	3	4	5	6	7	8	9	10
9. Nervioso/a	0	1	2	3	4	5	6	7	8	9	10
10. Ansioso/a	0	1	2	3	4	5	6	7	8	9	10
11. Deprimido/a	0	1	2	3	4	5	6	7	8	9	10
12. Furioso/a	0	1	2	3	4	5	6	7	8	9	10
13. De mal genio	0	1	2	3	4	5	6	7	8	9	10
14. Vigoroso/a	0	1	2	3	4	5	6	7	8	9	10
15. Cansado/a	0	1	2	3	4	5	6	7	8	9	10

¿Cómo te sientes hoy? (Escoge "Mal" o "Bien" y un número de la escala)

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Annex 2. Published paper for Study 2.

Aromatherapy Improves Work Performance Through Balancing the Autonomic Nervous System

Lin Huang[1,2] and Lluis Capdevila, PhD[2]

Abstract

Objective: This study analyzed the efficacy of aromatherapy in improving work performance and reducing workplace stress.

Subjects: The initial sample comprised 42 administrative university workers ($M_{age} = 42.21$ years, standard deviation = 7.12; 10 male).

Intervention: All sessions were performed in a university computer classroom. The participants were randomly assigned into an aromatherapy group (AG) and a control group (CG), and they were invited to participate in a specific session only once. They were seated in front of a computer. During the intervention period, some oil diffusers were switched on and were in operation throughout the session with petitgrain essential oil for AG sessions and a neutral oil (almond) for CG sessions. At the same time, participants completed a computer task on a specific Web site typing on their keyboard until they had finished it. The single times were different for all participants and were recorded on the Web site as ''performance time.''

Outcome measures: Before and after the intervention, participants completed anxiety and mood state questionnaires (the Stait–Trait Anxiety Inventory [STAI] and the Profile of Mood States [POMS]). Heart-rate variability (HRV) was measured before (PRE), during (20–25 min), and after (POS) the intervention to analyze autonomic nervous system regulation.

Results: The AG performed the Web site task 2.28 min faster than the CG ($p = 0.05$). The two groups showed differences in the following HRV parameters: low frequency ($p = 0.05$), high frequency ($p = 0.02$), standard deviation of all RR intervals ($p = 0.05$), and root mean square of differences ($p = 0.02$). All participants in all groups showed a decrease from PRE to POST for STAI ($p < 0.001$), Tension-POMS ($p < 0.001$), and Vigour-POMS ($p = 0.01$) scales.

Conclusions: Aromatherapy (inhaling petitgrain essential oil) can improve performance in the workplace. These results could be explained by an autonomic balance on the sympathetic/parasympathetic system through a combined action of the petitgrain main components (linalyl acetate, linalool, and myrcene). The final effect could be an improvement of the mental and emotional condition by a combination of reducing the stress level and increasing the arousal level of the participants in terms of attentiveness and alertness.

Keywords: aromatherapy, work performance, autonomic balance, heart rate variability, workplace stress, burnout

Introduction

Workplace stress and burnout are pervasive problems nowadays. They potentially affect employees' job performance and their physical and mental health.[1] Several studies have recently shown that the level of workplace stress and diseases are interrelated.[2] Studies have also proved that workplace stress disorders are associated with impaired workplace performance and hefty economic costs, as well as an increased risk of cardiovascular morbidity and mortality.[3] Chronic job stress can be associated with a range of debilitating health outcomes, including cardiovascular diseases, diabetes, depression and anxiety, musculoskeletal problems, or alcohol dependence.[4] Workplace stress also has

[1]School of Tourism, Kunming University, Kunming, China.
[2]Department of Basic Psychology, Autonomous University of Barcelona, Barcelona, Spain.

been found to affect job satisfaction, performance, and turnover intentions.[5] This is now a serious problem—around 40–50% of all work-related absences are related to stress. In the European Union, 350 million work days are lost because of stress-related problems, resulting in an overall cost of at least €20 billion every year. Thus, this problem has been noted by the European Commission.[2] In the United States alone, businesses lose $300 billion annually as a result of lowered productivity, days of absence, and healthcare costs due to stress.[4] Increasingly, experts call for the implementation of workplace health promotion programmers, as psychological problems, stress-related physical complaints, perceived work stress, and obesity have negative effects on workers' health as well as overall productivity. Thus, workplace stress-related illness is a serious issue, and job stress should be identified at an early stage so that preventive action can be taken.[4]

Many stress reduction–related methods have been investigated, and various approaches have been involved in workplace stress management. Aromatherapy is one of these approaches, thanks to its easy implementation and effectiveness.[6] It is used by an increasing number of people around the globe, and clinical research into this field is also expanding every year. This discipline has grown in importance as an area in alternative medicine with proven high efficacy in reducing stress and improving mood disorders.[7] Aromatherapy is an ancient tradition herbal medicine, and it was used in some ancient countries, such as Egypt, China, and India, thousands of years ago. It is a kind of natural treatment method utilizing the chemical structure and effects of plant essential oils, which when extracted from herbs, roots, leaves, flowers, and other plant parts can be used to treat various diseases.[8]

Aromatherapy has significant therapeutic potential because each essential oil has a unique combination of chemical compounds that interact with the body's chemistry and thereby affect specific organs, systems, and the body as a whole. For example, some studies have shown that certain essential oils decrease anxiety-related behavior and improve some illnesses in humans and animals.[9,10] Essential oils are the principal component of aromatherapy. They are concentrated volatile compounds formed as secondary metabolites in aromatic plants and are characterized by a strong odor. Their biological effects may be the result of the synergistic effect of all the molecules together or only of the main molecules found in larger concentrations in these oils.[11]

The essential oil can be absorbed into the body through three main channels: the skin, the digestive system, and the olfactory system. Sense of smell plays an important role in the physiological function of an individual because the olfaction response is immediate and it is directly extended to the brain; it seems to be related to emotional behavior.[12] The odor molecules of essential oils are transported to the brain by olfactory sensory neurons in the nasal cavity. Thus, they stimulate smell–memory work and influence memory, thoughts, and emotions. Even a small amount of smell molecules taken by respiration causes an indirect physical effect by activating smell memory or by infiltrating the blood.[13,14] The inhalation of essential oils triggers an olfactory response that leads to powerful mental and emotional behavioral changes, which address the body, mind, and spirit for mental, emotional, and physical wellness.[15] Some studies have shown that aroma has an important impact on attention level, cognitive performance, creativity, mathematical success, writing skills, motor skills, and increased perception and memory.[16]

The present study pays particular attention to the essential oil petitgrain (*Citrus aurantium* (*fe*); CA). CA belongs to a large family, *Rutaceae*, members of which are cultivated worldwide as fruit crops. Traditionally, populations in several countries have relied on preparations obtained from Citrus species to treat problems related to the nervous system, especially symptoms of anxiety or insomnia.[17] In folk medicine, CA is used for the treatment of sunstroke and gastrointestinal disturbances. It is also known to be a relaxant.[18] Recently, some studies have shown that CA has sedative and anxiolytic-like effects. Lehner et al. found that CA reduced the anxiety levels of patients, specifically women, when this oil was scattered in the lobby of a dental office.[19] Another study showed that CA (administered orally and derived from the petals and stamens of petitgrain oil) reduced the preoperative anxiety of patients scheduled for elective minor surgery.[20] CA has increasingly been used in several studies, and its chemical components have been described as linalool (20.2–27%), linalyl acetate (45–55%), and myrcene (1.3–12.12%).[21] Myrcene, a kind of monoterpene, is a component biologically active in the central nervous system.[17,21] The other main components—linalool and linalyl acetate—have sedative effects.[21,22]

The absorption of essential oil via inhalation may affect the autonomic nervous system (ANS) and induce the reaction of the limbic system, hypothalamus, and hormone system. It could affect both parts—the sympathetic nervous system and the parasympathetic nervous system—which function primarily in opposition to each other.[6]

Heart-rate variability (HRV) has become a popular research tool because it reflects changes in the cardiac autonomic regulation.[23] Hence, HRV is considered as a window to the ANS. HRV analysis is a valuable, simple, and noninvasive method for analyzing the continuous changes in the sympathetic–parasympathetic balance of the ANS. HRV refers to the variation in the time interval between consecutive heartbeats (the RR interval), and when there is variation at rest, it can be interpreted as a sign of healthy cardiac function.[24] This analysis has been established during the past few decades as a reliable tool to assess the status of the cardiovascular autonomic function. Thus, it has been frequently used in the analysis of physiological signals in different clinical and functional conditions.[25]

Taking all this into account, the purpose of this study was to analyze the efficacy of aromatherapy in improving work performance and reducing the workplace stress. Specifically, the effects of smelling CA during a simulated computer work session were examined to see if performance time when typing on a keyboard improved. At the same time, the possible effects of HRV and mood/anxiety state questionnaires were analyzed to explain the results in terms of sympathetic–parasympathetic balance, stress reduction, and changes in arousal or attention level.

Material and Methods

Participants

The initial sample comprised 42 administrative university workers ($M_{age} = 42.21$ years, standard deviation [*SD*] = 7.12; 10 male). All participants met the following inclusion

criteria: (1) aged between 26 and 55 years; (2) no clinical diagnosis of autonomic dysfunction; (3) no clinical diagnosis of respiratory, cardiovascular, endocrine, or systemic disorder; and (4) no problems with olfactory function. All participants consented to collaborate voluntarily and provided informed consent before participating in the study. The ethics committee of the university approved the study in advance.

Study design

A randomized controlled trial was used. The participants were recruited from university administrative staff through an institutional e-mail advertisement. All of them were used to sitting and typing in front of a computer for most of the working day. The participants were randomly assigned to the aromatherapy group (AG) or the control group (CG), and they were invited to participate in specific sessions for each group on different days. They were not aware of which group they were assigned to. Explanations were provided regarding the nature and purpose of the study: to investigate coping effects of plant fragrance on workplace stress. All participants were asked not to consume any food or beverages containing alcohol or caffeine after the evening of the day preceding the experiment. They were also asked not to do vigorous physical activity for 24 h before testing.

Aromatherapy and essential oils

The participants inhaled essential oils from four diffusers that allowed the smell to be dispersed fully throughout the room. Two kinds of oils were used, one for each group. The AG inhaled petitgrain oil (CA; Italian, Production batch: IF745, Neroly Co. Ltd, Hong Kong). The CA that was used was 100% pure, and it was diluted to 2%. The predominant constituents were linalyl acetate (45–55%), linalool (20–27%), and myrcene (1.3–12.12%). The CG inhaled sweet almond oil (*Prunus dulcis*, mill [PD]; American, Production batch: YA637; Neroly Co. Ltd). The predominant constituents were palmitoleic acid (60–68%), linoleic acid (17–30%), and palmitic acid (6–8%). PD is a vegetable oil and has a neutral smell.

Questionnaires

This study used three cognitive instruments. The first was a global ''Feeling Scale,'' asking ''how do you feel today?'' Participants had to choose one value on a 0–10 scale (where 0 = ''very bad'' and 10 = ''very good''). The second instrument was a short version of the Profile of Mood States (POMS).[26] Fifteen items measure mood states by five scales: the Tension scale, where the higher the score, the more tense the subject is; the Depression scale, where the higher the score, the more depressed the participant is; the Hostility scale, where the higher the score, the more hostile the subject feels toward others; the Vigor scale, a positive scale, where the higher the score, the more energy the subject has; and the Fatigue scale, where the higher the score, the more tired the participant feels. All five scales contain three items that are scored on a scale from 0 = ''nothing'' to 10 = ''very much.'' The third instrument was the State–Trait Anxiety Inventory (STAI),[27] which was used to measure the participants' pre- and post-intervention cognitive anxiety. The STAI scale comprises 20 items, and the participants were asked to rate the intensity of each symptom on a scale ranging from 0 = ''not at all'' to 3 = ''very much,'' resulting in scores ranging from 0 to 60. The higher the score, the more anxiety state the subject has.

HRV instruments

Participants' session ratings of continuous heart rate (RR or beat-to-beat cardiac intervals) were collected and analyzed with Fitlab® Team system (HealthSportLab.com, Barcelona, Spain). This consists of an application that runs in a mobile device (tablet or iPad) connected via Bluetooth with several cardiac chest bands, and connected via wireless with a remote server. The system allows synchronized recordings of all participants in each session to be performed and for the quality of data to be checked in real time. Participants are asked to remain at rest in the same position without speaking or making any movements, and HRV data are registered continuously for 5 min of natural breathing. This technology has been validated for detecting RR intervals at rest.[24]

Procedure

The participants were randomly distributed into six sessions (seven subjects each) on different days, three for the AG and three for the CG. All sessions were performed in a university computer classroom between 10:00am and 11:30am, and each participant attended a session only once. When the participants arrived at the computer classroom, they were asked to wear the cardiac chest band (Bluetooth connected) and to rest in a sitting position in front of a computer, as they would in their workplace. First, participants were required to fill out the STAI-State, POMS, and the Feeling Scale questionnaires. Second, HRV-Pre was tested for 5 min with the participants at rest (sitting, with the eyes open, placing the hands on their knees, with no activity, with natural breathing, and without speaking; see Fig. 1). Third, at the end of HRV-Pretest, the oil diffusers were switched on and remained operational throughout the session with CA for the AG sessions and PD for the CG sessions (Fig. 1A). At the same time, a researcher explained for about 15 min how to complete a neutral computer task on a specific Web site. This task was the same for all participants, and consisted of completing 10 standard forms. All participants began to complete this task individually, typing on their keyboard until they had finished it (step B–D in Fig. 1). The single times (B–D) were different for all participants, and were recorded on the Web site as ''Performance Time.'' A period of 5 min-HRV (Fig. 1C) was recorded at 20–25 min after the start of the inhalation period (A).[28] By 40 min after inhalation began (A), all participants had finished their Web site task, and their HRV was recorded for a period of 5 min (POST; step D–E in Fig. 1), under the same conditions as the HRV-Pretest. Finally, participants were asked to fill out the POST questionnaires (STAI-State, POMS, and the Feeling Scale).

HRV analysis

RR intervals from 5 min periods were analyzed in accordance with the recommendations of the Task Force of the

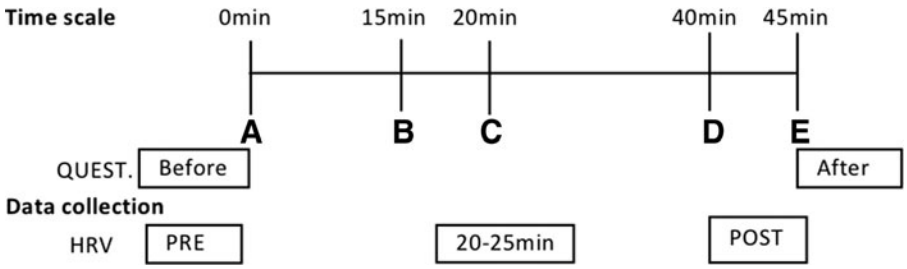

FIG. 1. Experimental procedures and data collection. Before: State–Trait Anxiety Inventory (STAI) and Profile of Mood States (POMS) questionnaires. PRE: 5 min HRV-Pretest. **(A)** Inhalation beginning. **(B)** Computer task beginning. **(C)** 5 min HRV ''20–25 min'' test. **(D)** Computer task finishing (individual ''Performance Time''). POST: 5 min HRV Post-Test (step **D–E**). After: STAI and POMS.

European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[29] For the time-domain analysis, the mean of RR intervals (RRmean), the standard deviation of all RR intervals (SDNN), and the root mean square of differences (RMSSD) of successive RR intervals were calculated. For frequency domain analysis, all RR series were resampled at 3 Hz using a cubic spline prior to the HRV analysis. The power spectrum of the resampled time series was estimated using a fast Fourier transform algorithm after removing the mean of the time series and multiplying the time series by a Hann window. The power of the very low frequency band (VLF) was estimated by integrating the power spectrum for frequencies <0.04 Hz. Accordingly, the power of the low frequency (LF) band was computed in the range 0.04–0.15 Hz, and the power of the high frequency (HF) band was computed in the range 0.15–0.4 Hz. Additional calculations included the LF/HF ratio, LF, and HF values expressed in normalized units (LFnu and HFnu).

Statistical analysis

Data are expressed as the mean ± *SD*. One-way analysis of variance (ANOVA) was used to detect possible differences between the AG and the CG in all parameters and variables of the study. An ANOVA according to a general linear model (GLM) was performed to show HRV differences between the two groups in terms of evolution throughout the three HRV stages of the session (PRE, 20–25 min, and POS). A power analysis was calculated ($\alpha = 0.05$), and the results ranged between 0.500 and 0.996 for the different significant parameters. All calculations were performed using the IBM SPSS Statistics for Mac OS v21 (IBM Corp., Armonk, NY). The significance threshold was set at $p < 0.05$. Calculation of HRV parameters was carried out with MATLAB.

Results

Among the 42 initial participants, five did not complete the study because they showed too many artefacts in the HRV recordings. In the AG, 20/21 participants completed the study; in the CG, 17/21 participants completed the study. There were no statistically significant differences between the AG and the CG with regard to age (AG: 43.0 ± 6.4 years old; CG: 43.2 ± 6.8 years old) or sex (AG: 20% male; CG: 31.6% male; see Table 1). No significant differences between the two groups were found for HRV control variables such as taking medication, doing physical activity, and drinking coffee.

The one-way ANOVA showed that the AG performed faster than the CG did: the performance time for the AG was 17.35 ± 3.13 min, and for the CG it was 19.63 ± 3.87 min ($p = 0.05$; see Table 1).

Table 2 shows the differences in the HRV parameters between the two groups at baseline (PRE), at 20–25 min from the start of the inhalation period, and after the inhalation period (POS). An ANOVA according to a GLM showed differences between the two groups in terms of evolution throughout of the three stages of the session (PRE, 20–25 min, and POS) for the following HRV spectral parameters: LF, such that AG showed a larger LF decrease between PRE and 20–25 min than CG did, and CG showed a larger LF increase between 20–25 min and POS than AG did ($p = 0.05$; see Fig. 2A); HF, such that AG showed a larger HF decrease between 20–25 min and POS than CG did, and CG showed an HF increase at this stage ($p = 0.02$; see Fig. 2B). Figure 2C and D shows the differences between groups with a trend to significance for HFnu ($p = 0.08$) and LFnu ($p = 0.08$). The VLF parameter also showed a trend toward statistical significance ($p = 0.058$). GLM analysis showed differences between the two groups for the time domain HRV parameters: SDNN ($p = 0.05$; Fig. 2E) and RMSSD ($p = 0.019$; Fig. 2F). There were no significant differences between the two groups for the ratio LF/HF, RR mean, and HR mean.

There are no differences in the questionnaires scorings between the two groups in terms of evolution from before (PRE) to after (POST) the session. However, there were significant differences for all participants, not group depending, between PRE and POST for the STAI ($p < 0.001$),

Table 1. Sex (%), Age, and Performance Time (*M* ± *SD*) for the Two Groups

Variable	*Control group* (n = *17*)	*Aroma group* (n = *20*)	p-*Value*
Age (years)	43.21 ± 6.80	43.00 ± 6.44	NS
Sex			
Male	31.6%	20%	NS
Female	68.4%	80%	
Performance time (min)	19.63 ± 3.87	17.35 ± 3.13	0.05

NS, no significance.

Table 2. HRV Parameters ($M \pm SD$) in the Three HRV Session Recordings (PRE, 20–25 Min, and POST) for the Two Groups

HRV parameter	*Control group* (n = *17*)	*Aroma group* (n = *20*)	*Total* (n = *37*)	p-*Value*
RRmean (ms)				
PRE	854.64 ± 118.91	870.10 ± 92.10	863.00 ± 104.03	<0.001
20–25 min	865.35 ± 114.52	884.85 ± 107.01	875.89 ± 109.40	
POST	896.88 ± 112.69	907.35 ± 103.02	902.54 ± 106.17[a]	
HR (bpm)				
PRE	71.50 ± 9.73	69.70 ± 7.65	70.58 ± 8.59[b]	<0.001
20–25 min	69.41 ± 7.99	67.83 ± 7.65	68.60 ± 7.76	
POST	67.77 ± 8.09	66.89 ± 7.27	67.32 ± 7.59	
SDNN (ms)				
PRE	45.64 ± 14.19	54.10 ± 21.76*	50.21 ± 18.91	0.05
20–25 min	50.47 ± 16.88	48.45 ± 16.84*	49.37 ± 16.65	
POST	57.76 ± 22.40	56.90 ± 23.96	57.29 ± 22.94	
RMSSD (ms)				
PRE	26.58 ± 8.50	33.30 ± 17.90*	30.21 ± 14.59	0.02
20–25 min	31.23 ± 8.37	32.65 ± 21.07*	32.00 ± 16.31	
POST	32.94 ± 11.96	32.20 ± 13.67	32.54 ± 12.74	
LF/HF (ratio)				
PRE	4.17 ± 4.21	4.36 ± 5.48	4.27 ± 4.87	NS
20–25 min	5.53 ± 6.04	3.04 ± 2.49	4.18 ± 4.59	
POST	5.73 ± 7.12	3.45 ± 1.88	4.50 ± 5.07	
LF (ms^2)				
PRE	1013.05 ± 1036.89	1222.05 ± 1426.21*	1126.02 ± 1250.01	0.05
20–25 min	990.94 ± 731.01	739.15 ± 605.55*	854.83 ± 668.74	
POST	1514.35 ± 1695.93	1025.20 ± 737.48*	1249.94 ± 275.31	
HF (ms^2)				
PRE	258.52 ± 156.12	498.30 ± 560.25*	388.13 ± 437.22	0.02
20–25 min	266.58 ± 165.24	576.45 ± 1066.77*	434.08 ± 798.28	
POST	346.29 ± 218.50	393.15 ± 367.22*	371.62 ± 304.88	
VLF (ms^2)				
PRE	779.00 ± 652.38	1258.25 ± 1052.01[c]	1038.05 ± 12.08	0.058
20–25 min	1167.76 ± 986.78	932.75 ± 663.68[c]	1040.73 ± 824.22	
POST	1815.76 ± 1949.89	1957.55 ± 2182.60	1892.40 ± 2051.62	
LFnu (%)				
PRE	71.11 ± 15.42	69.50 ± 17.92	70.24 ± 16.61	0.08
20–25 min	74.64 ± 14.81	66.20 ± 18.26[c]	70.08 ± 17.08	
POST	70.94 ± 20.81	73.10 ± 11.88	72.10 ± 16.37	
HFnu (%)				
PRE	28.22 ± 15.42	30.50 ± 17.92	29.75 ± 16.61	0.08
20–25 min	25.35 ± 14.81	33.80 ± 18.26[c]	29.91 ± 17.08	
POST	29.05 ± 20.81	26.90 ± 11.85	27.89 ± 16.37	

[a]Total differences for all sample: POST > PRE.
[b]Total differences for all sample: PRE > POST.
[c]Trend to significant differences between the two groups.
*Significant differences between the two groups.
HRV, heart-rate variability; HR, heart rate; SDNN, standard deviation of all RR intervals; RMSSD, root mean square of differences of successive RR intervals; LF, low frequency; HF, high frequency; NS, no significance; VLF, very low frequency.

Tension ($p < 0.001$), and Vigour ($p < 0.015$) scales. In all cases, the participants showed a decrease from PRE to POST.

Discussion

This study confirms the importance of aromatherapy as a way to improve quality of life and performance in the workplace. Inhaling CA in a simulated computer work session improved the performance time when typing at a keyboard, and it also produced some changes in HRV. Thus, the AG (inhaling CA) finished the task >2 min before the CG (inhaling PD), when doing the same task, which consisted of completing specific web forms. With the aid of the HRV analysis, this result can be explained by a combination of reducing the stress level and increasing the attention level of the participants.

A very common workplace task in all the world was chosen for this study. Typing with a keyboard in front of a computer for a full workday is a usual task in many jobs such as university staff, bank staff, or administrative staff in any company. The participants in this study were administrative university staff, and a non-specialized computer task was chosen: to complete a limited number of questionnaires and self-reports about the participant's own lifestyle. Thus,

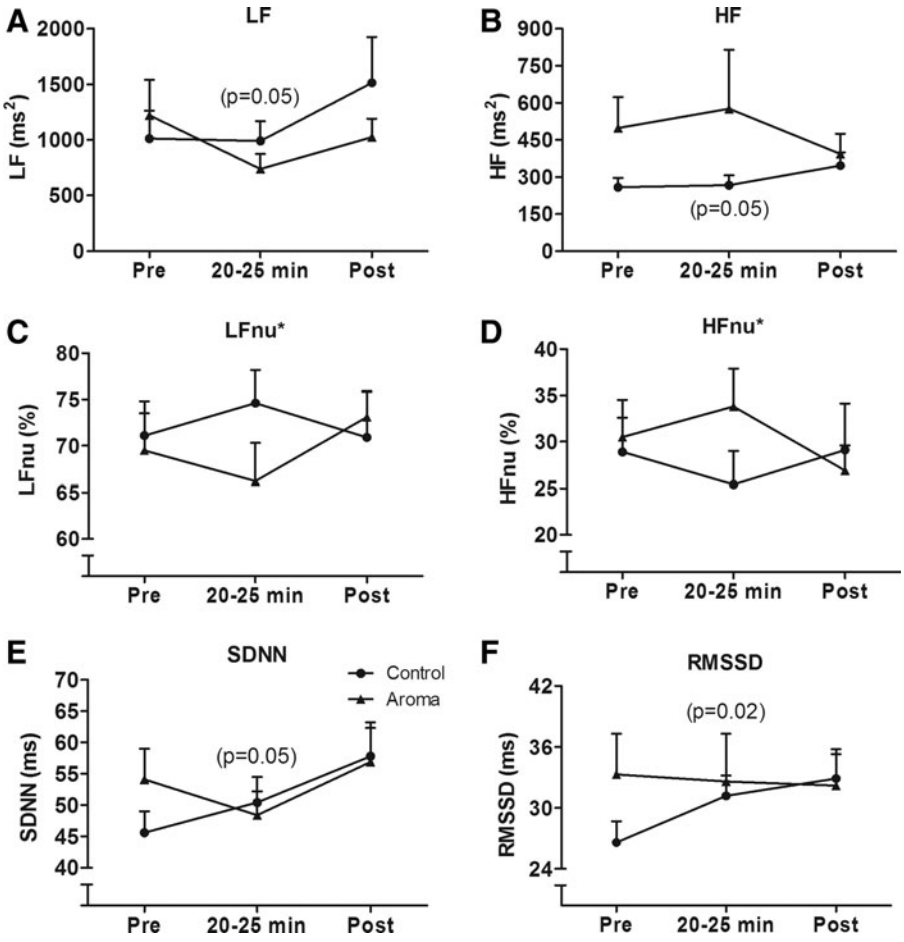

FIG. 2. Comparison of HRV parameters during the inhalation period between the two groups at baseline (PRE), at the 20–25 min period, and after the inhalation period (POS). **(A)** Low frequency (LF) band; **(B)** high frequency (HF) band; **(C)** LF values expressed in normalized units (LFnu; $*p = 0.08$); **(D)** HF values expressed in normalized units (HFnu; $*p = 0.08$); **(E)** standard deviation of all RR intervals (SDNN); **(F)** root mean square of differences of successive RR intervals (RMSSD).

there were no right or wrong answers, and all participants knew the answers. The best performance required thinking of the answers quickly and typing fast on the keyboard. All items had to be completed, after which the participant was asked to click on the last option of ''Save.'' An individualized report was provided to all participants about their lifestyle, and all of them completed the items with coherent answers. The participants were randomly assigned to two groups, so that they were balanced in terms of mental and typing agility. Hence, under these conditions, the performance improvement of the AG group (>2 min faster than the CG) must be attributed to the aromatherapy effects (inhaling CA).

The problem of job-related stress is currently increasing and is potentially affecting employee performance and personal health.[30] Studies have been carried out about relieving mental load in the workplace through strategies such as analyzing exercise effects on autonomic balance,[25] or the effects of aromatherapy in alleviating work-related stress.[31] A growing number of studies have described the efficacy of aromatherapy in this field.[32] However, few studies have examined the effects of aromatherapy directly on work performance through the autonomic balance. This study has contributed to this by using HRV analysis to explain the performance improvement in the AG by stress reduction due to the effect of aromatherapy.

This stress reduction could be explained by a specific effect on the ANS. For this purpose, this study analyzed HRV in three 5 min periods: before inhalation, 20 min after inhalation began, and after the inhalation period. The main HRV findings reveal significant differences between both groups in HF- and LF-related parameters. These indexes have been used to reflect primarily sympathetic and parasympathetic influences.[33] The larger LF decrease between PRE and 20–25 min in the AG could be explained by a specific effect of inhaling CA reducing the sympathetic activity during the task performance. Otherwise, the sympathetic effect could be effective during the last session period, where the CG shows a larger LF than the AG did. In this last session period (between 20–25 min and POST), the parasympathetic activity (showed by HF) is different in both groups. This same trend can be seen for the standardized parameters. Thus, the most important aromatherapy effect is the increase in parasympathetic activity (HF and HFnu increases) for the AG between PRE and 20–25 min, combined with a decline in sympathetic activity (LF and LFnu decreases) for this group in the same period. These changes could produce a sympathetic/parasympathetic balance of the ANS, improving attention control and facilitating performance. In addition, the significant decrease of the SDNN parameter in the AG during the task (PRE and 20–25 min) means less cardiac variability than the CG, and could be interpreted as an increase in arousal. This higher arousal could enable the AG to pay more attention to the task, thereby improving performance. Thus, inhaling CA could improve the mental and emotional condition in terms of attentiveness and alertness.

The study also analyzed the cognitive levels of anxiety and mood state as stress symptoms. However, no differences

were found between the two groups. Significant differences were found for all participants for anxiety scores (STAI) and psychological tension (POMS). They showed less anxiety values after the session, but these global results were expected because they had just performed a theoretical stressful task. More than one aromatherapy session may be needed to obtain more cognitive anxiety reduction in the AG. What is the mechanism of action underlying the performance improvement effect of inhaling CA? Aromatherapy could drive ANS activity toward a balanced state.[6] This balance could be explained by the combined action of CA's main components. Some of these components have a sedative effect, such as linalyl acetate and linalool, which could induce changes on the parasympathetic activity.[22] In line with this, Kuroda et al. found that HF power increased more significantly at 21 min after 6 min of olfactory stimulation with linalool.[34] In a similar vein, the present study found changes at 20–25 min after starting the inhalation period. Thus, CA seems to have an increasing effect on parasympathetic activity in the AG (HF and HFnu), which was clearly higher at the end of the session (POST). Meanwhile, the CG decreased HF and HFnu values in the POST period. Myrcene is another component in CA that could have some activation effects by stimulating the sympathetic activity.[15] Myrcene, a kind of monoterpene (1.3–2.12%), could possibly be related to the release of norepinephrine, a neurotransmitter linked with the sympathetic activity stimulation.[6] This could help to explain the increase in arousal levels in the AG. Similar results have been found with inhaling pepper oil, which induced an increase in adrenaline concentration, while inhalation of rose oil decreased the adrenaline level. The same stimulating effects of essential oils on sympathetic and parasympathetic activity have been reported, for example estragon oil or grapefruit oil. These essential oils, like pepper oil, consist of components such as limonene, pinene, or methyl chavicol, which possibly mediate the stimulating effect on sympathetic activity. Furthermore, alcohol such citronellol, geraniol, nerol, and patchouli alcohol were detected in rose oil and patchouli oil, which induced inhibition of parasympathetic activity.[35]

The olfactory system interactions could be another mechanism of action to explain the effects of CA through an interaction with central nervous system structures (e.g., hypothalamic, limbic, thalamic), which control the level of autonomic and behavioral arousal.[9] The olfactory neuroanatomy is intertwined, via extensive reciprocal axonal connections, with primary emotion areas, including the amygdala, hippocampus, and orbitofrontal cortex and other parts of the limbic system. The odor molecules of essential oils are transmitted to the brain by olfactory sensory neurons in the nasal cavity. Thus, they stimulate smell–memory work and influence memory, thoughts, and emotions.[36] Further studies are needed to evaluate the olfactory system interactions and another possible action mechanisms underlying the workplace stress and performance improvement effect of aromatherapy.

Conclusion

This study confirms that aromatherapy (inhaling petitgrain essential oil) can improve performance in the workplace. The most important aromatherapy effect was the increased parasympathetic activity for the AG between PRE and 20–25 min period, combined with a decline in sympathetic activity for this group in the same period. These changes could produce a sympathetic/parasympathetic balance of the ANS, improving attention control and facilitating performance. This balance could be explained by a combined action of the petitgrain main components (such as linalyl acetate, linalool, or myrcene). On the one hand, some of these components have sedative effects, which could induce changes on the parasympathetic activity. On the other hand, other components could have some activation effects by stimulating the sympathetic activity. The final effect could be an improvement of the mental and emotional condition by a combination of reducing the stress level and increasing the arousal level of the participants in terms of attentiveness and alertness.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Lluis Capdevila, PhD
Department of Basic Psychology
Autonomous University of Barcelona
08193 Bellaterra (Barcelona)
Spain

E-mail: lluis.capdevila@uab.cat