ACTIVATING EFFECT OF AROMATHERAPY INHALATION WITH *Citrus hystrix* (KAFFIR LIME) LEAVES OIL

ALISA STEPHANIE RASION

FACULTY OF SCIENCE AND NATURAL RESOURCES UNIVERSITY OF MALAYSIA SABAH

2018

ACTIVATING EFFECT OF AROMATHERAPY INHALATION WITH *Citrus hystrix* (KAFFIR LIME) LEAVES OIL

ALISA STEPHANIE RASION

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF SCIENCE AND NATURAL RESOURCES UNIVERSITY OF MALAYSIA SABAH

2018

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: ACTIVATING EFFECT OF AROMATHERAPY INHALATION WITH *Citrus hystrix* (KAFFIR LIME) LEAVES OIL

IJAZAH: SARJANA SAINS (KIMIA INDUSTRI)

Saya ALISA STEPHANIE RASION, sesi 2016-2018, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini adalah hak milik Universiti Malaysia Sabah
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/):

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan
☐	TIDAK TERHAD	

Disahkan Oleh,

_____ _____

ALISA STEPHANIE RASION
MS1521001T

(Tandatangan Pustakawan)

_____

Tarikh : 1st October 2018

(Prof. Madya. Dr. How Siew Eng)
Penyelia

DECLARATION

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

27th September 2018

Alisa Stephanie Rasion
MS1521001T

CERTIFICATION

NAME	:	**ALISA STEPHANIE RASION**
MATRIC NO.	:	**MS1521001T**
TITLE	:	**ACTIVATING EFFECT OF AROMATHERAPY INHALATION WITH *Citrus hystrix* (KAFFIR LIME) LEAVES OIL**
DEGREE	:	**MASTER OF SCIENCE (INDUSTRIAL CHEMISTRY)**
VIVA DATE	:	**9TH AUGUST 2018**

CERTIFIED BY;

SIGNATURE

SUPERVISOR

PROF. MADYA. DR. HOW SIEW ENG _____

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to the God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully. I would like to express my sincere gratitude to my advisor Prof. Madya. Dr. How Siew Eng for the continuous support of my master study and research, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis and finally I am extremely grateful to my parents and to my friends for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

Alisa Stephanie Rasion

27th September 2018

ABSTRACT

Citrus hystrix leaves oil has a scent with unique nuances because it is rich in natural constituent citronellal which possesses soothing properties and has the potential to be used as aromatherapy. However, there have been relatively few published about the *C. hystrix* leaves extract and its efficacy as aromatherapy. This research was aimed to investigate the effects of *C. hystrix* leaves oil on human behavioural parameters after inhalation. *C. hystrix* essential oil was obtained from fresh leaves by steam distillation and the oil yield obtained was 0.14% with pale yellow in colour while the chemical profile of *C. hystrix* leaves volatile oil was analysed using the gas cromatography-mass spectrometry (GC-MS). It was found that the citronellal is the highest percentage (85.00%) followed by citronellol (4.75%), citronellyl propanoate (2.74%), delta-3-carene (1.15%), geranyl acetate (0.84%), (E)-ß-ocimene (0.69%), linalool (0.42%), terpinolene (0.40%), myrcene (0.35%), camphene (0.25%), ß-pinene (0.24%), and α-Phellandrene is the lowest percentage (0.05%). 3-(4,5-dimethylthiazol- 2yl)-2,5-Diphenyl tetrazolium bromide (MTT) test was used to measure cytotoxicity of the oil and from this research it was found that the essential oil of *C. hystrix* (leaves) showed less toxic to the normal human liver cells (WRL 68) with the highest value of IC_{50} (40.23±14.86 ug/mL) where it required a higher concentration of the *C. hystrix* (leaves) oil to inhibit the activity of the cells by 50% while the low dose concentration of paclitaxel reduced the percentage viable cells (0.0016±0.0003 ug/mL). There were 40 respondents participated this survey which were randomly assigned into two groups, control group (treated with virgin coconut oil (VCO) since this oil has no scent and it is typically used as a carrier oil) and the *C. hystrix* oil group and each group consists of 20 respondents. Visual analog scale (VAS) was used for this survey for the subjects to rate their emotional condition in order to assess subjective behavioural arousal in terms of relaxation, vigour, calmness, attentiveness, mood and alertness after inhalation. Data obtained in this study were analyzed using Mann-Whitney-U-Test, Wilcoxon Signed Rank Test, and Spearman's Rho Correlation. Based on the Mann-whitney U Test result, it showed that *C. hystrix* oil gave arousal effects in relaxation, vigorous and attentiveness and this data was supported by Wilcoxon signed rank test data for beginning and ending time where the data of *C. hystrix* oil category were significantly different in score of effects at the beginning and ending observation data for relaxation, vigorous and attentiveness rated by respondents. From this finding, *C. hystrix* oil gave arousal effects in relaxation, vigorous and attentiveness and these were supported by the Spearman's Rho Correlation test where relaxation had positive medium correlation with variable vigorous, $r = 0.47$, $n = 20$, $p < 0.05$, and variable vigorous had positive strong correlation with variable attentiveness, $r = 0.74$, $n = 20$, $p < 0.05$. The *C. hystrix* leaves oil has been experimentally shown to have a positive effect on relaxation, vigorous and attentiveness than subjects in the control group and these results provide evidence for its use in aromatherapy for the relief of depression and uplifting mood in humans and this might be due to higher content of citronellal in *C. hystrix* leaves oil and it is safe to be used.

ABSTRAK

KESAN PENGAKTIFAN AROMATERAPI SECARA SEDUTAN DENGAN MINYAK PATI DAUN CITRUS HYSTRIX (LIMAU PURUT)

Minyak pati daun Citrus hystrix mempunyai aroma yang unik kerana kaya kandungan semulajadi citronellal yang mempunyai sifat yang menenangkan dan mempunyai potensi untuk digunakan sebagai aromaterapi. Walau bagaimanapun, hanya sedikit yang diterbitkan berkenaan ekstrak daun C. hystrix dan keberkesanannya sebagai aromaterapi. Kajian ini bertujuan untuk mengkaji kesan minyak pati daun C. hystrix terhadap parameter perilaku manusia selepas sedutan. Minyak pati C. hystrix diperolehi daripada daun segar dengan penyulingan wap dan hasil minyak yang diperolehi adalah 0.14% dengan warna minyak kuning pucat manakala profil kimia minyak pati daun C. hystrix dianalisis dengan menggunakan gas kromatografi spektrometri jisim (GC-MS). Peratus sitronellal didapati adalah tertinggi (85.00%) diikuti oleh sitronellol (4.75%), sitronelil propionate (2.74%), delta-3-carene (1.15%), geranil asetat (0.84%), (E)-ß-ocimene (0.69%), linalool (0.42%), terpinolene (0.40%), myrcene (0.35%), camphene (0.25%), ß-pinene (0.24%), dan peratus α-phellandrena adalah terendah (0.05%). Ujian 3-(4,5-dimethylthiazol- 2yl)-2,5-Diphenyl tetrazolium bromide (MTT) digunakan untuk mengukur sitotoksisiti minyak dan daripada kajian ini didapati bahawa minyak pati C. hystrix (daun) kurang toksik kepada sel hati manusia yang normal (WRL 68) dengan nilai IC_{50} (40.23±14.86 ug/mL) yang tinggi dimana ia memerlukan kepekatan minyak pati C. hystrix (daun) yang tinggi untuk menyekat aktiviti sel sebanyak 50% manakala paclitaxel dengan dos kepekatan yang rendah mengurangkan peratus sel yang baik (0.0016±0.0003 ug/mL). Terdapat 40 orang responden yang menyertai kajian ini dan disusun secara rawak kepada dua kumpulan iaitu kumpulan kawalan (dirawat dengan minyak kelapa dara (VCO) memandangkan minyak ini tidak mempunyai aroma dan biasanya digunakan sebagai minyak pembawa) dan kumpulan C. hystrix dan setiap kumpulan terdiri daripada 20 responden. Skala analog visual (VAS) digunakan untuk kaji selidik ini bagi subjek menilai keadaan emosi mereka untuk menilai ransangan tingkah laku subjektif daripada segi relaksasi, kecergasan, ketenangan, perhatian, mood and kepekaan selepas penyedutan. Data yang diperolehi di dalam kajian ini dianalisis dengan menggunakan Mann-Whitney-U-Test, Wilcoxon Signed Rank Test, dan Spearman's Rho Correlation. Berdasarkan hasil analisis ujian Mann-whitney U, ia menunjukkan bahawa minyak C. hystrix memberikan kesan ransangan dalam relaksasi, kecergasan dan perhatian dan data ini disokong oleh data Wilcoxon Signed Rank Test bagi masa awal dan akhir di mana data bagi kategori minyak C. hystrix terdapat perbezaan yang signifikan di dalam skor kesan bagi data pemerhatian masa awal dan akhir untuk relaksasi, kecergasan dan perhatian yang dinilai oleh responden. Daripada kajian ini, minyak C. hystrix memberikan kesan rangsangan dalam relaksasi, kecergasan dan perhatian dan ini disokong oleh Ujian Rho Korelasi Spearman di mana relaksasi mempunyai korelasi sederhana positif dengan kecergasan, $r = 0.47$, $n = 20$, $p < 0.05$, dan kecergasan mempunyai korelasi positif yang kuat dengan perhatian, $r = 0.74$, $n = 20$, $p < 0.05$. Melalui eksperimen ini, minyak pati daun C. hystrix memberi kesan yang positif terhadap relaksasi, kecergasan dan perhatian daripada subjek di dalam kumpulan kawalan dan keputusan ini membuktikan bahawa penggunaannya di dalam aromaterapi dapat

melegakan kemurungan dan menaikkan mood manusia dan ini mungkin disebabkan oleh kandungan citronellal yang tinggi di dalam daun minyak pati C. hystrix dan ia selamat digunakan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/SYMBOLS

Rpm	Revolutions per minute
g	Gram
Kg	Kilogram
L	Liter
mL	Mililiter
µm	Micrometer
mg	Miligram
m	Meter
mm	Milimeter
eV	Electronvolt
µg	Microgram
µL	Microlitre
%	Percentage
˚C	Degree Celsius
CO_2	Carbon dioxide

nm	Nanometre
WRL68	The human hepatic cell line
DMEM	Dulbecco's Modified Eagle Medium
PSG	Penicillin-Streptomycin-Glutamine
FBS	Fetal bovine serum
NMDA	N-methyl-D-aspartate (receptor that is used to control the synaptic plasticity and memory function)
IC_{50}	Inhibitory concentration by 50%
L-NAME	N(ω)-nitro-L-arginine methyl ester (induced hypertension)
Ca^{2+}	Calcium ions
±	Standard deviation
≥	Greater than or equal to
<	Less than
>	Greater than

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Introduction

Aromatherapy is defined by Sense of Smell Institute (SSI) as the therapeutic effects of aromas on physical conditions such as digestive problems and menstrual disorders as well as psychological conditions such as chronic depression (Balchin, 2006). In a modern aromatherapy, both essential oils and their aromas are used for balancing, harmonizing and promoting the health of body, mind and spirit where the effects of an aroma can be instantaneous including both direct and indirect psychological effects even thinking about a smell may have a similar effect to the smell itself. However, some evidences showed that inhaled or dermal applied essential oils enter the blood stream and in relevant molecular, cellular or animal models, exert measurable psychological effects, indicates that the effects are primarily cabenefits.

Extraction obtained from mostly basic plant parts such as stems, seeds, flowers, resins, leaves, barks, roots, or fruit rinds produced essential oils which are highly concentrated substances (Rao and Pandey, 2006). Essential oils flavour and their therapeutic or odoriferous properties often used in foods, medicines, and cosmetics. There are three ways essential oils can be absorbed including through the olfactory and respiratory systems (vapour inhalation), transdermally through lotions or compresses involving massage and during bathing, or orally, through consumption of essential oils in capsules or as additives to food or medicinal preparations, for example but it is more to the herbal medicine than aromatherapy (Perry, and Perry, 2006). *Citrus* essential oil is the good source of aromatherapy and it was the most popular source of perfume and fragrance essences because of its good sour and sweet tastes, pleasant and refreshing aroma, as well as extensive growing areas worldwide.

There are several plants of *Citrus* rich in essential oil often used in the anxiety treatment and among these plants are orange, *Citrus sinensis* (Faturi *et al*., 2010), *Citrus aurantium*, bergamot (Carvalho-Freitas and Costa, 2002; Pultrini *et al*., 2006), *Citrus limon*, lemon (Ceccarellia *et al*., 2004) and these essential oils are often used as aromatherapy agents for relieving and for reducing stress and depression which have been used topically for their antibiotic and antiviral properties for over the years (Setzer, 2009; Cooke and Ernst, 2000). Since the genus *Citrus* essential oil showed positive emotional effects in studies with humans and demonstrated anxiolytic as well as sedative effects in experimental models of anxiety and hence, it could be a great alternative (Pimenta *et al*., 2012). Hence, *C. hystrix* (Kaffir lime) leaves will be used for this research which is from the same genus *Citrus* and the *C. hystrix* leaves that have a strong fragrant lemon scented. It was previously reported that heart disease, dizziness and indigestion, as well as physical nourishment, could be treated by *C. hystrix* which used as herbal medicine (Abdul, 2013). *C. hystrix* leaves have antimicrobial properties and commonly been used for cooking and its essential oil or oleoresin for aromatherapy (Siti *et al*., 2016).

C. hystrix is an indigenous plant in Southeast Asia including Malaysia, Indonesia and Thailand. *C. hystrix* from Rutaceae family has it's own aroma and commonly used as spices in cooking and medicinal herbs. *C. hystrix* has a bumpy fruit with a rough skin while the leaves are an aromatic herb which used broadly in adding a distinctive aroma and flavour to food. There are two types of essential oil made from *C. hystrix*, one from the peel and the other from leaves. The *C. hystrix* leaves contain oleoresin such as citronellal which give it aroma. The major component in the *C. hystrix* leaves is citronellal (Kawiji, *et al*., 2015). Basically, the higher content of geraniol, citronellal hydrate, citronellal, linalool, linalyl acetate, the more delicate its aroma (Kawiji *et al*., 2015). The major component of the ethyl acetate extract from leaves of *C. hystrix* is citronellal (73.08%) and that from hydrodistillation is also citronellal (100%) while the major components of the hydrodistillated essential oils from *C. hystrix* peels are limonene (16.85%) and cironellal (3.57%) whereas citronellal (2.80%), limonene (0.31%), and pinene (0.13%) were major components of essential oil of the ethyl acetate extract (Megawati, 2009). Exposure to *C. hystrix*

aroma produced the effect of reducing "disguise iritation" which is evaluated based on cognitive evaluation and it indicates that exposure to the *C. hystrix* aroma produces a positive emotive response which may impact clarity of mind and health (Megawati, 2009). *C. hystrix* could be a great alternative and has the potential in research and commercialization for the solution in insect repellent, aromatherapy, and spa practices, making shampoo, beauty product as well as antioxidants compound (Nurhani *et al.*, 2013).

Since *Citrus hystrix* leaves oil has a scent with unique nuances because it is rich in natural constituent citronellal which possesses soothing properties and has the potential to be used as aromatherapy. Steam distillation was used in this research because steam distillation produces more citronellal from the leaves than the other methods where previous results reported that citronellal was the major component in *C. hystrix* leaves oil with the highest percentage (80.04%) (Srisukh *et al.*, 2012) compared to other techniques such as solvent extraction, hydrodistillation, supercritical fluid extraction, pressurised liquid extraction (PLE), and maceration extraction where the results report that the percentage of citronellal is 74.8%, 66.85%, 1.41%, 25.87%, and 25.66% respectively (Tinjan and Jirapakkul, 2007; Loh *et al.*, 2013; Haiyee and Winitkitcharoen, 2012; Kawiji *et al.*, 2015).

Citronellal gives therapeutic benefits including antiviral, antimicrobial, tonic, vasodilators, a hypotensive, calming, antipyretic, sedative, and spasmolytic (Bouayed and Bohn, 2012) and at the various activity levels, *C. hystrix* leaves oil has excellent activity against many respiratory bacteria (Srisukh *et al.*, 2012). Thus, it can be used as air purifying to clean the air naturally using the air purifier. Steam distillation is used to extract essential oil for this research because it is economy for small and medium scale industry, cheap for a basic level process, as well as there is no changing properties of oils produced by this method. The oil never spoil in this method since the steam reduces the boiling point of a particular component of the oil and this method also faster than other methods (Kumar, 2010). Steam distillation is the oldest

method and simplest method to produce essential oil using leafy materials for commercial purpose.

Before the essential oil of *C. hystrix* can be tested on humans, toxicity test should be conducted first. Safety tests begin with acute toxicity testing where a single dose of the test compound is given to animals and this test is focusing on determining the range between the two possible doses which is no adverse effect and the dose that is life-threatening. Toxicity divided into two types which are acute or chronic and these types are based on the number of exposures to a poison as well as the time it takes for toxic symptoms to develop. Short-term exposure with a high dose causes acute toxicity while the repeated or long-term exposure with low doses causes chronic exposure. MTT test is commonly used for cytotoxicity testing because of their rapid, standardized and sensitive in determining if a material contains significant quantities of hazard components. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay determines the cells activity where it is based on the conversion of MTT into formazan crystals by living cells (Meerloo *et al.*, 2011). MTT test can be extrapolated the results to toxicity to mammals since it uses tissue culture from cell (Tunjung *et al.*, 2015). MTT assay is more suitable technique for the application to toxicity test for human and because for this reason MTT test is used for this research to determine the *C. hystrix* oil toxicity.

1.2 Significance of the Study

Hectic daily tasks of modern life can give a stressful life and people nowadays tend to take drugs of pain killer to stop the pain or the uncomfortable symptom that linked to depression, anxiety and stress but drugs are toxic to the body and have the side effects compare to the natural molecules such as those found in essential oils. An essential oil is multi-dimensional and filled with homeostatic intelligence where it has the capability in restoring the body to healthy balance and if there are changes in body conditions, the oil will adapting, increasing or reducing blood pressure as needed, stimulating or suppressing enzyme activity as needed, energizing or relaxing

as needed (Stewart, 2005). Treatment with essential oils is safer and fewer adverse effects.

1.3 Scope of the Study

There have been relatively few published about the *C. hystrix* leaves oil and its efficacy as aromatherapy. Therefore, the present study of *C. hystrix* leaves oil is to investigate the potential of inhalation with *C. hystrix* leaves oil as aromatherapy on healthy human emotional responses where the scope of this research is to study the positive effect of *C. hystrix* leaves oil on stimulation of human relaxation, vigour, calmness, attentiveness, mood and alertness which linked to individual performance. Stimulating oil gives expected effect on mind and body. The benefits of this research for users are to improve the emotional balance and to improve overall attitude which increases the life quality and health. Users can effectively perform their tasks and make a maximum efficiency.

Research Objectives

Objectives of this research are:

a) To determine the chemical components of *C. hystrix* oil using Gas cromatography-mass spectrometry.
b) To evaluate the toxicity of *C. hystrix* oil.
c) To evaluate the stimulating effect of *C. hystrix* aroma on humans such as relaxation, vigour, calmness, attentiveness, mood and alertness.

CHAPTER 2

LITERATURE REVIEW

2.1 Aromatherapy

Aromatherapy is practicing the use of natural oils and which also referring to essential oils therapy. In aromatherapy, the main therapeutic agents are essential oils which are highly concentrated substances extracted from plants and these oils are good to apply in the treatment of mental and physical balance which give stress relief, revive and revitalize the individual for a next day's duty (Ali *et al.*, 2015). Aromatherapy is the holistic practice of preventative approach and as an active technique to use during the stages of acute and chronic illness. The use of aromatic plants has been around since Neolithic times and these aromatic plants have been used for beauty treatment, perfuming, medicinal practices, cleansing, and ritual bathing as well as religious ceremonies (Stiles, 2017).

In fragrance psychology field, there is a various number of group for study including perfumes (e.g. colognes, fine fragrances,), cosmetic and toiletry products with scent (e.g. hair-care, fragranced skin-care, and hand care products), environmental fragrances (e.g. room fresheners, potpourri) as well as aromatherapy products (essential oils) (Graham, 2000). Fragrance psychology is the perception of individual odour where these odours are likely to affect a various field of human psychological functioning, ranging from perception and mood to cognitive processes and attitude as well as the changes in humans induced by smelling the fragrances of essential oils (Lenochova *et al.*, 2012; Sugawara *et al.*, 2013). The psychology includes the product behavioural effects on the user including others perceptions (personality, the appearance, and projected image) self-perception (moods, self-image, confidence, self-esteem, and attitudes), interpersonal attraction (sexual attraction and liking) and in psychology field also includes motivations or reasons for using or purchasing a product which relating scent to differentiate situation and

lifestyles and to link individual fragrances with particular personality aspect and finally it access therapeutic effects including mental and physical particularly to relevant aromatherapy products (Graham, 2000).

Application of single or combined essential oils including diffusing the oils into inhaled air, using in massage oil or adding to bathwater. The inhalation of these essential oils molecules triggers the olfactory nerve which sending the messages to the brain's limbic system including the cognitive, memory, and emotion that are said to stimulate the physiological responses for examples lavender promotes relaxation and eucalyptus relieves congestion. The utilization of extracted aromatic plant essence gives the aromatherapy benefits where the treatment outcome could be achieved by conveying the odours to the central nervous system via the skin and olfactory system. The odours are dispersed in the blood and circulate to different organs which can prevent and treat the central nervous system diseases and for this reason, this application makes the aromatherapy as compatible therapies where the essential oils as the major therapeutic agents can treat several diseases (Lee *et al.*, 2011).

The major methods used in aromatherapy that use the essential oils are including inhalation, local application, and baths. These oils penetrate the human skin surface and once the oils are in the system, they regulate themselves and fix the affected area or malfunction site and this type of therapy uses various permutation and combinations to get relief from numerous illness such as depression, insomnia, headache, indigestion, muscular pain, skin ailments, respiratory problems, urine associated complications, swollen joints and so on (Ali *et al.*, 2015). Aromatherapy can be classified as massage aromatherapy, olfactory aromatherapy cosmetic aromatherapy, medical aromatherapy, and psycho-aromatherapy.