A report for the methods used for the extraction of lavender essential oil and its uses.

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2016

Word count: 5377

Abstract

Lavender belongs to the *Lamiaceae* family. There are four main types of lavenders: *lavandula angustifolia, lavandula latifolia, avandula stoechas*, and *lavandula x intermedia*. In this paper, the main focus is based on *lavandula angustifolia* and the chemicals inside it. For those chemicals, linalool, linalyl acetate, 1,8-cineole, and camphor will be discussed. From those chemicals, there are several properties of this flower, such as cosmetic, medicine, cuisine, and aromatherapy. Also chemical analytical techniques, namely gas chromatography, and mass spectrometry are going to be emphasized, in addition to solid phase extraction. In the end, further interpretation for the experiment about the simple extraction of lavender essential oil with direct experience and procedure is given.

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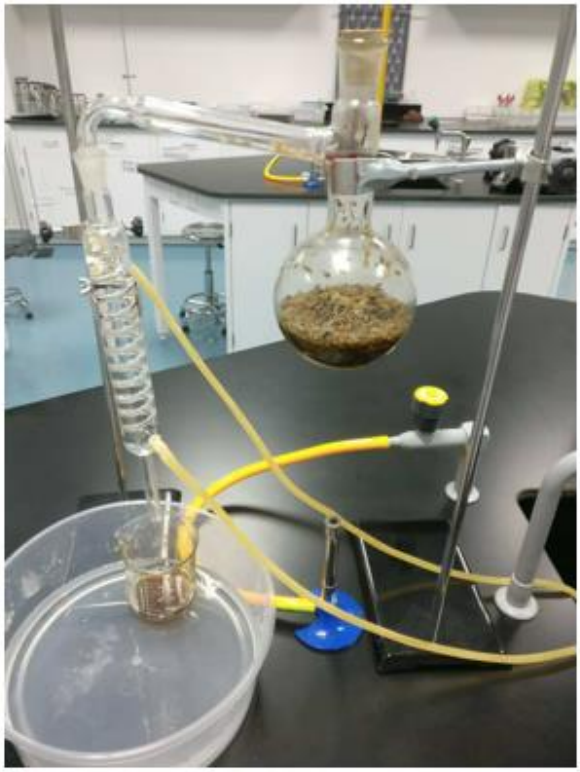
Introduction:

"Lavender essential oil is produced, usually by steam distillation", is discussed by (M.A.Cavanagh and M. Wilkinson 2002, p.1). Their research indicates that solid phase extraction is needed as well. Moreover, detailed chemical analysis needs two more advanced techniques: gas chromatography and mass spectrometry. Those two ways are used for identifying different chemicals, applying for their chemical structure and relative molecular mass.

A picture taken of the experiment



Once it was favored and applied into cosmetic products during Victorian times. Lavender oil, nowadays, is mixed with different other essential oils and aromachemicals (Lis-Balchin 2002, p.16). Lis-Balchin's book 'The genus *Lavendula*' indicates this from her research. Moreover, still asserted by (Lis-

Balchin 2002, p.16), the genetic instability of plants are caused by numerous different variations such as fertilizer, latitude, and geometric position etc; hence, the chemical composition differs greatly, with one of the most aromatic oil being derived from the flowers (McGimpsey and Porter, 1999), retrospected by (M.A.Cavanagh and M. Wilkinson 2002, p.1). Findings convey that there is no need to do research about which type of Lavender essential oil contains certain compounds such as linalool and linalyl acetate in large quantities. However, different types of oil show different activities against several bacterials; meanwhile there is no single type that shows the highest antibacterial activity against all types of bacteria (Cavanagh and Wilkinson 2005, p.2). Antibacterial activity does not directly relate to concentrations of different chemical components according to Cavanagh and Wilkinson (2005, p.2). Tracing back to my own project, the overall goal is to extract essential oil from lavender flower with high purity.

Main Contents:

Lavender *(lavendula)* belongs to the *Lamiaceae* family, and *Nepetoideae* subfamily as explained by (Lis-Balchin, 2002) in the book called "The genus *lavendula*". This idea is accepted by Cavanagh and Wilkinson (2002), who wrote that lavender "belongs to the family *Labiatae (Lamiacae)*". However, as suggested by a Chinese team, "*(Lamiaceae)* belongs to the Mint family" Chen, and Yang (2016, p.2). But in fact, according to Scully (2008), *lamiaceae* and mint families have the same biological terms. Further arguments by Lis-Balchin (2002), showed that lavender is actually difficult to be defined as a single group but rather a divergent one. Despite its variable features among different assortments: large differences in height (from one decimeter to one meter), different styles of leaf, and the color of flowers (Ellagance Pink, blue, and purple etc) (Lis-Balchin, 2002). Its nectary lobes are between the opposite directions of the flower shows the characteristics of *Lamiaceae* family (Scully 2008. p1), but the flower spike are "[…] either in an opposite decussate arrangement (each pair of flower whorls at right angles to the pair above or below) or an alternate spiral arrangement". Still recounted by Lis-Balchin (2002), lavender is originally cultivated from France and was spread all over the world because of its beauty and its different uses. Examples of which include:

- cosmetic
- medicine
- cuisine
- aromatherapy (developed later)

In this paper, medicine and aromatherapy will be the main topics to be discussed.

At the same time, "Lavenders fall into four main categories", as discussed by (M.A.Cavanagh and M.Wilkinson, 2002).

- *Lavandula latifolia*
- *Lavandula angustifolia*
- *Lavandula stoechas*
- *Lavandula x intermedia*

In this paper, *Lavandula angustifolia* will be the main type of lavender to be discussed.

Lavender—essential oil

"Essential oils are complex mixtures of many different aromatic compounds", directly stated by Cavanagh and Wilkinson (2002 p.2). Also different types of Lavender have relatively different functions. For example, *L. angustifolia* is used in the perfumery and cosmetic industries while the high camphor containing lavenders are used as insect repellents and for other non-perfumery uses (Cavanagh and Wilkinson 2005). Nevertheless, somehow differently shown by European Medicines Agency (2012), *lavendula angustifolia* essential oil does have clinical effect on human body. According to European Medicines Agency (2012), many clinical activities can be reached from *L. angustifolia* oil, such as blood flow rate and nerve activity. At the same time Verma et al. (2010) shows the composition of *lavendula angustifolia* essential oil mainly contains linalyl acetate and linalool, which is just almost the same as results presented by Lis-Balchin (2002); however, there are many different minor constituents which are not emphasized by Lis-Balchin, (2002). Namely alpha-terpineol, geranyl acetate, and caryophyllene oxide (some more chemicals I did not indicate) (Verma et al. 2010, p.3). What cause these differences? In fact, some differences do occur in both oil composition and in the reported therapeutic uses for different species; mentioned by Cavanagh and Wilkinson (2005). Moreover, this idea is extended by McGimpsey and Porter (1999) in another paper of Cavanagh and Wilkinson's, namely that oil composition is mainly determined by the genetic make-up of each cultivar, and also can be affected by the distillation process. Consolidated more accurately by experiments by Rmili et al. (2014), there is a difference of yield produced between hydrodistillation and microwave-assisted hydrodistillation (around 1.5% higher). What is the reason for that difference? Known adequately in scientific area, mass spectrometry and gas chromatography are the main ways to determine chemical compositions as emphasized by Cobice et al. (2015), Rmili et al. (2014), and Rompp et al. (2013). As another piece of information, the analysis of mass spectrometry and gas chromatography is already very difficult. With different distillation results; hence, the final composition will relatively be different, which directly matches the Cavanagh and Wilkinson (2002) indication.

As I used Lis-Balchin's (2002) interpretation as my final conclusion. The main constituents in lavender oil are quite complex (Lis-Balchin,2002):

- linalool

- linalyl acetate
- 1,8-cineole
- beta-ocimene (usually both cis- and trans-)
- terpinen-4-ol
- camphor

In this paper, explanations for some of the chemicals above are provided, namely linalool, linalyl acetate, 1,8-cineole, and camphor.

Lavender essential oil

Used in folk medicine since ancient Romans and Greeks (Cavanagh and Wilkinson, 2002), lavender essential oil is famous for its therapeutic properties according to Lis-Balchin (2002). Moreover, "Essential oils are often used in alternative medicine as analgesic and anti-inflammatory remedies.", which directly indicated by Takaishi, et al. (2012). Meanwhile, the extracted oil has sedative, carminative (smooth muscle relaxing), and anti-depressive properties, in addition to its recognised anti-microbial effects according to (Cavanagh and Wilkinson, 2005). Also effective for anti-fungal, burns, and insect bites as mentioned by (Grieve 1931; Gattefosse, 1937) then re-mentioned by (Cavanagh and Wilkinson 2002, p.1). However, "the specific compounds that confer the effects of essential oils and the molecular mechanisms are largely unknown", as interpreted by (Masayuki Takaishi, Fumitaka Fujita et al. 2012).

However, the lavender essential oil is toxic towards certain enzymic reactions (Khosravi and Sendi 2013, p.49), which is apparently different from the paper of Cavanagh and Wilkinson (2005) mentioned: no direct relationship between anti-microbial activity of different types of lavender oil and their major constituents. After deeper investigation, according to Choi et al. (2003), essential oil has the potential to kill the insect, greenhouse whitefly, *(Trialeurodes vaporariorum)* not only in its mature form but also for its nymphs and eggs due to the color change of the egg and ecdysis of the nymph. More specifically and intensively, (Khosravi and Sendi 2013, p.350) digestive enzyme activity of lavender oil is concluded from the remained larvaes, namely (Xanthogaleruca luteola). After larvaes' midguts are tested, results shows that

alpha-amylase activity decreased significantly. Which of them is wrong? In fact, both of them are correct. Through previous experiments, we know that lavender essential oil kills different insects. Meanwhile, unlike other basic pesticides, lavender oil does not have direct effects on humans and does not cause direct damage to the nervous system of insects. It works on the enzyme inside the insects: alpha-glucosidase and alpha-amylase (Khosravi and Sendi 2013, p.353).

1, 8-cineole and camphor, which is the best anesthetic chemical?

Lavender essential oil has been used for a long time, one of the main functions—most commonly used—is its anesthetic property; however, some people do not think so. Where is the evidence and reason hidden beneath this chemical property?

Through investigation by Takaishi, Uchida, Fujita, and Tominaga (2014), Monoterpenes, such as camphor, and 1,8-cineole, are well-known for their analgesic, anti-inflammatory, and anesthetic effects.

"Lavender essential oil acts via the limbic system (in human brain), especially working towards amygdala and hippocampus for promoting sleep and relaxation." (Cavanagh and Wilkinson, 2002). At the same time, Gorji, Ashrataghi, Habibi, Charati (2015) indicated that the oil could pass the blood–brain barrier; hence, it can interact with the central nervous system receptors. In addition, still indicated by Gorji, Ashrataghi, Habibi, Charati (2015), the cholinergic system might play a role for these medical effects. Based on research by aforementioned sources, there are lots of messages that show 1,8-cineole and camphor working as some of the main components for those medical effects above. Although the mechanisms of antinociceptive effects of monoterpenes need further explanation, different TRP channel family are believed to be involved. This idea was asserted by Milanovic, Milijasevic, Ubavic, and Mikov (2015 p.166). "Transient receptor potential (TRP) channels respond

to a wide variety of sensory stimuli, including temperature, nociceptive compounds, touch, osmolarity, and pheromones." This idea was from (Takaishi, Uchida, and Fujita 2012, p.1), which is one of the original forms of research focusing on investigation of the effect of the mechanism of chemical components on animal bodies. Nevertheless, a different interpretation was given by Koulivand et al. (2013). According to Koulivand et al. (2013), the mechanism of the lavender oil in the nervous system was suggested by dopaminergic neurotransmission, which leads to antioxidant effect. Apart from those explained above, Cavanagh and Wilkinson (2005), the mechanism of psychological action of lavender essential oil should be similar to the benzodiazepines and to enhance the effects of gamma-aminobutyric acid in the amygdala (Tisserand, 1988). Since there is no authorized mechanism given, not a single explanation can be judged. However, different mechanisms play different functions, which can be implied from their paper. For example, the mechanism of dopaminergic neurotransmission is used for spatial memory deficits (Koulivand et al. 2013), and Alzheimer's disease (Koulivand et al. 2013; Soheili et al. 2015). Meanwhile, through aromatherapy, the interpretation of mechanism is accepted by Gorji, Ashrataghi, Habibi, Charati (2015), and his team gives a further hypothetical explanation: "Some effect happens on aroma, which increase the gamma amniotic effects that in turn generate some sedative and tranquilizing outcomes." After inhaling of essential oil, enzymes, and ionic channels will be affected; hence, generates "multiple gamma amino butyric effects that end in brain stimulation, anxiety decrement, anti-depressive effects, and increased blood circulation in the brain." 1,8-cineole has abilities to activate hTRPM8 (a receptor relates to anti-nociception) and inhibit hTRPA1 (a nocisensor related to irritative reaction); hence, shows anesthetic and analgesic effect from another paper of (Takaishi, Uchida, and Fujita 2012, p.3).

Simultaneously, camphor is known to do the same compared with that of 1,8-cineole; however, camphor may not be a good analgesic compound "because it causes a warm and hot sensation, probably through TRPV1 activation." (Takaishi, Uchida, and Fujita 2012). From other research, which shows contradictory results, pure ingestion of camphor might even have a danger on the human body. MacKinney et al. (2015) mentioned a case of a 52-year-old Nepali man ingested around 10grams of pure camphor; results in grand mal seizures. This idea is explained by Lamnauer (2010), large amount of essential oil without dilution can cause dermatitis (skin irritation) and a series of problems since large amount of essential oil is consider as narcotic poison.

Linalool and perillyl alcohol—anti-cancerous chemicals

Extended even further, since the proliferation of scientific investigation, more advanced chemical properties have been found; anti-cancerous property is one of them. In these branches, there is a large potential maintained because many anti-cancerous mechanisms remained unknown.

The anti-cancerous effect of lavender essential oil has been discussed for several decades; however, even right now the mechanism about how do the components in lavender oil help to inhibit differentiation of tumor cells is unknown. This idea can be found in many papers, which talk about experiments, conclusions and hypotheses. "A number of studies have shown the ability of chemopreventive phytochemicals to increase the sensitivity of cancer cells to conventional anti-cancer drugs", accepted by (Ravizza, Gariboldi, and Moltni 2008, p.1).

At the same time, perillyl alcohol was found to be a powerful chemotherapeutic agent, targeting for several cancers such as pancreatic, breast, and liver cancer after many different animal studies in oncology, mentioned by (Belanger 1998, p.1). Exhibiting the increased apoptosis of tumor cells, Perillyl alcohol successfully inhibits the growing cycle of tumor cell, indicated by Buchbauer, in Balchin's book "The Genus *Lavendula*". However, according to Chen et al. (2015), an experiment was done by 18 patients who took soft gelatin capsules with 250mg perillyl alcohol and 250mg soybean oil. The results were disappointing. Through clinical observation: nausea; vomiting; anorexia; satiety; eructation. In addition to avoid fatigue. It is true that perillyl alcohol does have a chemotherapeutic effect. How to help patients to absorb the chemicals is the main problem. Instead, Chen et al. (2015) suggested another method to deliver perillyl alcohol. This alternative method is presented in Brazil, namely nasal inhalation with recurrent malignant glioma. Further interpretation shows that it works more easily to access to intracranial tumor site since nose-to-brain transport increases drug access. Meanwhile, vascularized epithelium of nasal mucosa with its high blood flow rate, large surface area, and low enzyme

amount (with comparison to other parts of bodies) shows the easily intake of the drug more successfully and quickly. Moreover, perillyl alcohol will not damage the normal cell (Belanger 1998, p.1). This idea above was proved by Belanger, which was proved by "the hamster experiment". The conclusion showed perillyl alcohol stops the continued growth of tumor cells in hamsters by stimulating of apoptosis based on in vitro findings. "There was no observable toxicity to the hamsters", asserted by (Belanger 1998, p.2). Also accepted by Chen et al. (2015), there was no damage to the hamster's liver, kidney, and pancreas. Later, the anti-cancer effect was investigated by Gould and Haag, in addition to Mills's team with different animal studies, and the result was: "the monoterpene caused a redifferentiation of the tumor cells", directly summarized by (Belanger 1998, p.4). In general, perillyl alcohol inhibits the incidence (percentage of animals with tumors) and exhibited increased apoptosis of tumor cells (Buchbauer 2002, p.131). But through the experiment by Chen et al. (2015), after a longer time injecting perillyl alcohol, less rate of decrease for neoplastic liver foci is then observed. Also hypothesized by (Chen et al. 2015, p. 1581), it is possible for perillyl alcohol to act differently in the early and late stages of carcinogenesis. After all, stye potential of chemotherapeutic agent needs to be explained even further; whether, mixing different essential oil, as suggested by (Seo et al. 2015), or combining with hybridoma (antibodies from animal experiment) (Jones et al. 2015, p. 239), the result remains unknown.

Despite the study of perillyl alcohol, there are also some papers that show the anti-cancer activity of linalool. Mei Yin and Yi Ling (2014) used WST-1 assay analysis to test the cytotoxic effects of linalool towards the effect of cancer cells. Result showed linalool inhibiting the growth of cancer cells with a range of certain sizes. Strongly suspected by another team, showing a different result by using animal studies, "linalool only moderately inhibits (tumor) cell proliferation", summarized by this team; meanwhile, combination of linalool with doxorubicin showed cytotoxic and pro-apoptotic effect in cell lines of cancer cells (Ravizza, Gariboldi, and Moltni 2008, p.2). Moreover, further experimental results reached a big divergence. From Mei Yin and Yi Ling (2014), in vitro wound-healing assay on breast epithelial tumor cell, there was a decrease of tumor cells because of the presence of single linalool. At the same time, a Chinese team proved the anti-cancer activity of linalool, in addition to that of linalyl acetate. From their "Cytotoxicity Assay", they showed linalool and linalyl acetate had potent cytotoxicity against typical cancerous type, which

is prostate cancer cell lines (Yunqi, Chen, and Yixin 2016, p.4). However, “increasing evidence from in vitro and animal models indicates that combining dietary-derived phytochemicals with conventional antineoplastic drugs is probably the most promising application", retorted by Ravizza, Gariboldi, and Moltni (2008, p.1).

Experimental techniques:

Derivation of those chemical properties is based upon the chemical analysis, namely gas chromatography and mass spectrometry. What are the advantages and limitations of them and how do scientists vanquish those limitations will be explained.

Gas Chromatography

Testing different substances can be somehow accepted by Davies, (1989). He mentioned different types of organic compounds, such as monoterpenes and sequiterpenes. These are separated and identified, mostly, by gas chromatography (Davies, 1989).

In the meantime, McDonald's team (2011) mentioned when gas chromatography coupled with mass spectrometry, many different compounds can be separated and measured quickly and accurately. This idea is even extended further explained by Kupiec (2004). According to Kupiec (2004, p.305), gas chromatography is better suited for testing small molecules.

However, this idea is somehow challenged by Oliver Fiehn (2000). The compounds that are being tested should be thermostable (Fiehn, 2000 p.1). Such ideas are questioned by Anderson and Armstrong (2005). A new class of ionic liquid monomers, after free radical reactions, can provide a more durable and robust stationary phase through cross linking processes (Anderson and Armstrong, 2005 p.1).

Another aspect from Soares et al. (2016), his team investigated another method called iconic liquids (ILs). When separation of solvents during solid phase extraction (mentioned below), ILs were applied into the process and increase

both the efficiency and accuracy for separating of different organic compounds in a more environmental-friendly way.

Still indicated by Fiehn's team (2000): the metabolites of plant, such as sugars, amino acids, and hydroxy acids are not volatile. At this point, McDonald's team (2012) gave a clear answer, although some targeted substances, such as steroids, will decompose since they are not intrinsically volatile, pre-analytical derivatization are provided before gas chromatography and mass spectrometry (GA/MS).

According to McDonald et al. (2012), excreted steroids will be separated from salts and condensed by solid-phase extraction (SPE). A solid phase extraction is simply the distribution of the typical chemical between a solid and the liquid, no matter whether the chemical is absorbed by the solid or or penetrated by the outer surface of the solid (Simpson, and Wells 2000, p.3). Hence, there will be an equilibrium for the chemical distribution, and we use other methods to filter the chemical from the solid (Simpson, and Wells 2000, p.4). After that, some charged moieties—excreted steroid forms as glucuronide and sulfate conjugates—will be eliminated by enzyme. Meanwhile, still indicated by McDonald et al. (2012), free hydroxyl groups, somehow matches hydroxy acids mentioned by Fiehn et al. (2000), mostly ketones will be altered and modified to become less polar and more volatile. Thus, these analytical substances can be used for further experiments (GC/MS).

Furthermore, the direct derivatization of reducing sugars, Fiehn's team used fructose and glucose as examples, "leads to a number of different peaks related to cyclic and open-chain structures of these hexoses that cannot be completely controlled by altering reaction conditions", which directly emphasized by Fiehn et al. (2000 p.2). This problem is somehow answered back by McDonald et al., they said an oximation step can reduce this effect since R-keto acids such as pyruvic acid are protected against decarboxylation. Meanwhile, Schauer et al. (2005) aptly retorted the idea from (Fiehn et al. 2000). They clearly declared the metabolite profiling can be identified unambiguously be GC with complex metabolite preparations with composite structure.

Mass Spectrometry

Mass spectrometry imaging (MSI), one of the most active fields in mass spectrometry (Rompp et al. 2013) is widely used in various territories, such as neuroscience, biochemistry, and clinical trails (Shariatgorji et al. 2013). This idea is concreted by Eshghi et al. (2012, p.1) because MALDI is an effective tool favored by its fast and accurate determination of many molecules. Meanwhile, Wu et al. 2014, stipulated: "More than thirty ambient ionization methods have been developed in the last eight years", which is proved by Alberici et al. (2010), Chen et al. (2009), and Harris et al. (2011) and this idea had been presented in Wu et al's paper already. According to Rompp et al. (2013), matrix-assisted laser desorption/ionization (MALDI) imaging can work at atmospheric pressure. Simultaneously, Wu et al. (2014) mentioned more developed ambient ionization techniques to make MSI happen under standard atmospheric pressure and outside the mass spectrometer in addition to the untreated samples. However, MALDI does have limitations. According to Eshghi et al. (2012), MALDI lost its accuracy because of detector sensitivity and chemical noise compromise.

From another aspects, Cobice et al. (2015) mentioned that MSI can be used to identify drugs and its endogenous compounds. Matrix-assisted laser desorption/ionization (MALDI) is one of the ionization technologies, closely related to mass spectrometry, which can be set for experiments, (The Yost Research Group 2015). However, there is a contradictory result showed by Eshghi et al. (2012). Namely, they ignore the low abundance of molecules from biological and clinical samples.

According to The Yost Research group 2015, MALDI is a microprobe technique that helps to generate chemically selective images. For sample, preparation—in order to prevent degradation of it—the analyte will be covered into a liquid nitrogen-isopentane bath and snap-frozen quickly (Jehl et al. 1981), which mentioned by Shariatgorji et al. (2013). Cutting the analyzed sample and making it into slice, attached it whether to a stainless steel plates or indium-tin-oxide glass slides (Shariatgorji et al. 2013), scientists coat the sample with a suitable MALDI matrix, namely help the sample to absorb UV light more easily

(The Yost Research Group 2015). The sample surface interacts with matrix; thus, extract analytes from sample tissue and incorporate them into matrix crystals (Rompp et al. 2013).

These processes of sample preparation are mainly related to organs from animals. It cannot prove the accuracy of biological molecules—as what Eshghi et al. (2012) emphasized above—since no connection and instruction shows to help analyze biological samples. Although according to Wu et al., (2014), ambient ionization imaging technique scan be applied for both 2D and 3D images. His team indicated MALDI is not considered as an ambient ionization imaging technique; hence, it requires sample preparation. After all, the accuracy of MALDI is provided by Eshghi et al. (2012). From the comparative experiment. An alternative method, namely targeted analyte detection (TAD). In this experiment, some known analytes were added into the solution in order to increase the concentration of those certain molecules above the noise level, which directly reduces the interference of noise level and increases the sensitivity.

Also the mass spectrum of an element can be used to calculate relative isotopic masses and abundances (Ryan and Norris 2014, p.446). "Peak intensities in mass spectra can be calibrated for analyte quantitation", which was directly indicated by (Rompp 2014). Timm et al., 2008 used peptide as an example. According to Timm et al. 2008, the composition of peptide, though not the only factor, affects peak intensity. In other words, peak intensity depends on peptide ionization efficiencies, and "the sensitivity of a mass spectrometer varies between peptides." Moreover, samples (peptides) are desorbed or vaporized by a heater and ionized by electrons by applying a laser beam (Rompp et al. 2013); hence, accelerated into the magnetic field and separated based on the mass/charge ratio (Hardinger 2006, p.1). "Ionization occurs externally to the mass spectrometer and those ions, not the entire sample", which was directly indicated by (Cooks et al. 2006) and emphasized again By Wu et al. (2014).

Peak intensity is one of the data analyzing processes after mass spectrometry imaging has shown.

The mass spectrum is quite difficult to be analyzed because of its complexity. According to Timm et al. (2008), who used peptide as an example, calculating target intensities for each peptide (analyte), protein will be identified correctly, digested perfectly, and the variable modification will be ignored. However, realistically, it is not going to work that perfectly. Noise peaks present in the mass spectrum (Yao et al. 2014); the changing base line of the mass spectrum (Scherbart et al. 2007 p.1); typical peptides present in more than one spectrum (Timm et al. 2008) all make the peak extraction and analysis really difficult. Because of those (specific or non-specific?)reasons, scientists come up with many different ways to overcome those variables. According to Scherbart et al. in 2007 and 2008, normalization is necessary since the amount of peptides in each spectrum is unsure. His team used two different mass spectrum for comparison. After that process, (informal) they can take account of the unmatched peptides and differences in the overall sensitivity of the detector by applying different equations. At the same time Yao et al. 2014 mentioned another way for analysis. His team divided the mass spectrum in different groups because the noise level is altered from different mass ranges in a single peptide spectrum. After doing so, they classified those peaks into three groups. Group A mostly has strong signal peak; group B contains some noise peaks and the peaks—related to typical chemicals—with low intensities; group C are mainly noise peaks. Moreover, the data in the chosen range of mass firstly arrayed in an ascending order and applied for further equations for determination to decide which data belong to noise peaks or chemicals. Hence, both methods work differently to improve the accuracy of further analysis of mass spectrometry.

Procedure:

The original solution is made by mixing ethanol and water inside the conical flask (increasing the boiling point of the solution). It was connected by a condenser with two rubber tubes, and one of the rubber tubes was linked to the water faucet to allow circulation of water between the condenser and outside surrounding. After that dry lavender flowers are added into the conical flask with certain quantity to make sure the percentage of chemicals inside the solution is high enough to be detected. The flask is then heated by bunsen burner. Vertical distance between the conical flask and bunsen burner is controlled carefully to prevent flowers being pushed into the glass tube of the flask and resulting in the blocking of the condenser by the external heat. After the mixed solution—ethanol and chemicals in lavender—was collected, filtration is applied (with wet filter paper), which is a slow process. With the comparison of the filtrated solution, there was an obvious color change—from dark brown to light yellow—also with the separation of small liquified residues remained on the filter paper.

A picture of the residue of the extracted flower

A picture of the dry flower

Conclusion:

Although the experiment is completed successfully, certain details need to be improved to make it better.

flower is pushed into the neck of conical flask

For example, the time taken for the final filtration was too long, far more than expected before. It could caused by some liquified residues within the dry flower. Separation of residues and solution is needed. According to Zarchi and Friedler (2012), alum can be used. However, still from Zarchi and Friedler (2012), various polyaluminium chloride coagulants (PACls) can be applied as well. Through the comparison between PACls, and alum, PACls has higher efficiency for water filtration. But obviously, there are chemical reactions between PACls and compounds in lavender essential oil. Hence, alum is recommended in this reaction. Another method, according to Reungoat et al. (2012), activated carbon filtration and ozonation can be applied for separating plant effluents with limited dissolved organic carbon removal, just matches the removal of liquified residue without removing essential oil solution as I mentioned before.

Meanwhile, continuously evaporating those volatile compounds during the time of filtration directly decreases the purity of my final filtrated solution. According

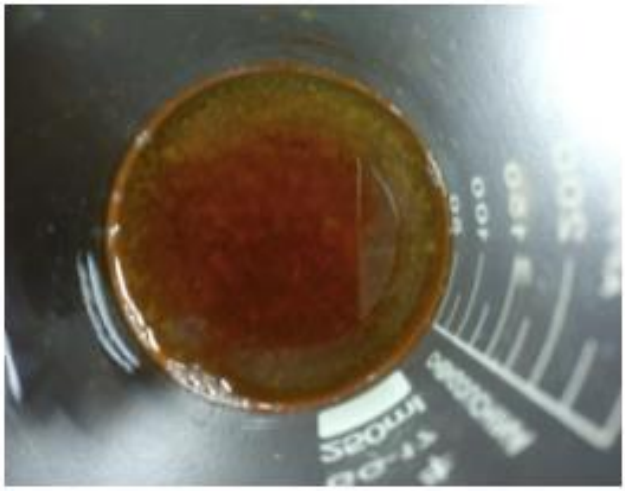
to Meng et al. (2016)—which is somehow similar to Reungoat's idea but more specific—low-concentration ozonation can be applied to extracellular polymeric substances (EPS), and most of EPS are organic substances. Through treatment of ozone gas, a great result from extraction efficiency of (EPS) is shown. However, still from Meng et al. (2016), polysaccharide concentration only had a slightly decrease at the end of the ozonation process. But thankfully, I do not need to analyze polysaccharides for my extracted solution of lavender oil. Hence, the result of experiment will not be damaged.

A picture of rough filtrated oil

A picture of refined oil

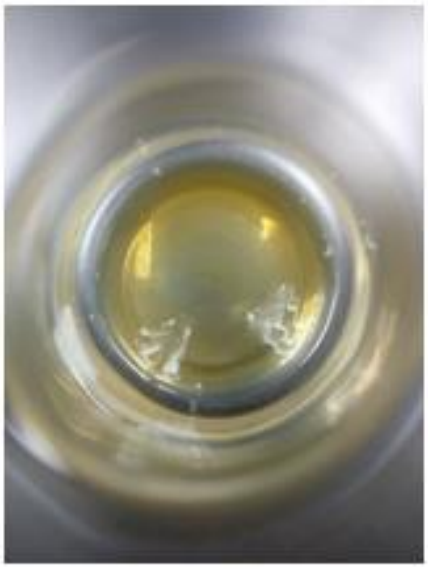
In addition, another way of filtration technique is covered by membrane technique. According to Nacheva et al. (2016), polycyclic aromatic hydrocarbons by membrane bioreactor (MBR), is used to form fiber membrane to separate alkene, namely naphthalene (NAP) and phenanthrene (PHE) as model compounds. The technique just matches lavender oil filtration, since lavender oil is used as aromatic compounds (Lis-Balchin 2002). If this technique can be applied to filter organic alkene, could we extend this technique further for separation of other organic compounds? The results still remain unknown due to no highly advanced equipments can be set or used.

liquified residue remains in the rough filtered solution



In addition, the solvent before is ethanol, which does not affect the results of the experiment and no extra damage of the filtered solution. However, more effective solvents can be applied. According to Paradiso et al. (2016), lactic acid and glucose are used as solvents, in addition of UV spectrophotometry for dissolving the phenolic compounds from virgin olive oil. Meanwhile, Durantini et al. (2016) mentioned, enzymic hydrolysis of 2-naphthyl acetate is applied by α-chymotrypsin, N,N-dimethylformamide (DMF), and water. Thus, multiple solvents applied to experiment is normal obviously. Hence, what occurs when a mixed solvent is coupled with another experimental techniques remains unknown; more analysis is needed.

final filtered solution remains clean



A picture of ethanol

The flower chosen is from Xin Jiang, which is one of the hottest places in China with the highest light intensity. Would this affect the different chemical composition from this lavender flower? According to Cavanagh and Wilkinson (2005), oil composition is mainly determined by the genetic make-up of each cultivar. Also, it can be determined by numerous different variations such as fertilizer, latitude, and geometric position (Lis-Balchin 2002, p.16). This idea is further extended by Nacheva et al. (2016). DNA glycosylases catalyze the release of methylated bases. This can cause DNA demethylation, and base excision repair pathway. The same as change the genetic composition of DNA. As what Jones et al. (2015) emphasized, namely cell meiosis is a change in DNA. Thus, further affects the oil composition of lavender.

Evaluation

Overall, this whole project is based on planning around my topic, and the apparatus chosen.

Extraction is the first and final decision of the topic due to its testability and affordability. Other advanced equipments were not available for a high school student. Meanwhile, many different kinds of flowers have been considered. In fact rose, as lavender, one of the most common essential oil plants, was considered at that time. However, extraction efficiency between rose and lavender are quite different. In the meantime, lavender has much higher extracted efficiency than rose does.

The heating and refinement processes are two of the main weaknesses of this project. More time for planning was needed. (connect with the sentence either before or after). Without careful design, only a big conical flask was applied; hence, water or oil bath cannot be set due to the size differences—the flask is bigger than the beaker—between two glassware equipment. Although according to Gelosa and Sliepcevich (2016), water condensation can be a problem, from the equipment aspect, as long as water bath and conical flasks are set up separately. There will be no regorging of water, causing the flask to break. Moreover, microwave irradiation can be used (Trivedi, and Kumar 2014).

Since there was insufficient time, only a bit of the crude solution is extracted, and even less for the final oil. In the meantime, traditional refining process contains degumming, bleaching and deodorization (Wafti. N et al. 2011, p.1). For this project, only simple filtration is used.

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