Perfiles aromáticos de aceites esenciales de cuatro variedades de albahaca en taiwán y estudio preliminar de su sensación aromaterapéutica

Aroma profiles of essential oils of four basil varieties in taiwan, and the preliminary study of their aromatherapeutic sensation

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Resumen

La albahaca dulce (Ocimum basilicum L.), la albahaca verde (Ocimum basilicum L. cv. green), la albahaca morada (Ocimum basilicum L. var. purpurascens) y la albahaca clavo (Ocimum gratissimum L.) presentan fragancias similares lo que ha ocasionado que las personas en general las consideren como la misma planta a pesar de que desde su taxonomía se establecen diferencias. Sin embargo, son ligeramente diferentes, al igual que sus aceites esenciales. Este estudio identifica las diferencias entre estos cuatro tipos de plantas en dos aspectos: primero, sus composiciones químicas; y segundo, su aroma. Los aceites esenciales de estos cuatro tipos de plantas se extrajeron mediante destilación al vapor. Los componentes de los aceites esenciales se analizaron y compararon mediante Cromatografía de Gases acoplada a Espectrometría de Masas (GC-MS). Profesionales en aromaterapia también evaluaron los aceites esenciales en su aroma y llenaron un cuestionario de preguntas cerradas. El resultado muestra que la albahaca verde y la albahaca morada son muy similares en términos de composición química y olor y pueden considerarse la misma planta; el methyl chavicol es el principal componente presente entre sus aceites esenciales. Mientras tanto, la albahaca dulce, la albahaca verde, la albahaca morada y la albahaca clavo presentan en general diferentes componentes químicos y en algunos casos presentan componentes químicos similares que varían en el porcentaje de peso de sus aceites esenciales.

Palabras clave: Ocimum basilicum, Ocimum gratissimum, albahaca, aceites esenciales, GC-MS, composición química.

Abstract

Sweet basil (Ocimum basilicum L.), green basil (Ocimum basilicum L. cv. green), purple basil (Ocimum basilicum L. var. purpurascens), and clove basil (Ocimum gratissimum L.) smell similarly and have been taken as the same plant by many for a long time. However, they are slightly different, and so are their essential oils. This study identifies the differences between these four types of plants in two aspects: first, their chemical compositions, and second, their aroma. Essential oils of these four types of plants are extracted by steam distillation. The components of the essential oils were analysed and compared using gas chromatography-mass spectrometry (GC-MS). Aromatherapists also evaluated the essential oils on their aroma and filled out a questionnaire of closed-ended questions. The result shows that green basil and purple basil are very similar in chemical composition and smell and can be considered the same plant; methyl chavicol is the main component present in its essential oils. Meanwhile, sweet basil, green basil, purple basil and clove basil generally have different chemical components and, in some cases, have similar chemical components that vary in the weight percentage of their essential oils.

Keywords: Ocimum basilicum, Ocimum gratissimum, basil, essential oils, GC-MS, chemical composition.

1. INTRODUCTION

The genus Ocimum, or its common name basil, is one of the most abundant genera of the Lamiaceae family (Sajjadi, 2006). Many cultivars of basil, which vary in size, colour and aroma are used as culinary or medicinal herbs (Dudai et al., 2020; Morales & Simon, 1996; Simon et al., 1999). The basil that is commonly used as a flavour is typically known as sweet basil, for example, in the Italian dish pesto (Formisano et al., 2021). In Taiwan, basil is a widely used herb. It is one of the essential spices in traditional cuisine (Erway, 2015). In kitchens, basil is also used for medicinal purposes and its essential oils (Evans, 1996; Patel et al., 2018). For example, Taiwan has seen the rise of growing herbs to extract essential oils; however, most farms lack understanding and experience in extracting essential oils (Zhang et al., 2010). After extraction, the content of the essential oils and their chemical composition might affect their usage (Ali et al., 2015). Thus, it is imperative to clarify the contents of those essential oils. Under the influence of planting conditions and environmental factors, the same type of plants growing in different places will have a varying chemical composition (Khalid, 2006; Da Silva et al., 2021).

In 5000 B.C., Indian discover a type of basil with a luxurious scent. They treated this plant as the avatar of the goddess Tulsi, and named that plant as such. The locals treated this basil as the god of grass and used it in the worship of their god Vishnu. They also use it in traditional Indian medicine Ayurveda (Javanmardi et al., 2002). Three thousand years ago, the Egyptian queen Cleopatra used essential oils for skin care and gave her body a pleasant scent. It is known that the smell of essential oils can affect someone's mind and feelings (Oumeish, 2001). In 1922, when the tomb of Egyptian pharaoh Tutankhamun was excavated, archaeologists learned that the Egyptians used galbanum, cinnamon, frankincense and cedar to preserve the bodies. Today's science confirmed that mummies, after almost three thousand years, show the remarkable antimicrobial properties of essential oils (Balasubramanian, 2015). Ayurveda, which has been practised continuously in India for nearly five thousand years, also describes the many uses of aromatic plants for religious and medical purposes (Mukherjee et al., 2012). In 1910 scientist René-Maurice Gattefossé accidentally burned his hand in a laboratory experiment, and there happened to be lavender oil nearby. He immediately put his hand into it. He felt his pain relieved, and his wound recovered well without a scar (Gattefosse, 2012). René-Maurice Gattefossé conducted experiments in military hospitals during World War I (1914-1918) and began describing aromatherapy in 1928 as a branch of science and therapeutics (Micozzi, 2018). Through Gattefossé, it is proven the effect of essential oils based on scientific evidence. Besides the effect on the physical body, basil also shows successful clinical practice on long-term psychiatric patients. In 1923 Gatti and Cajola noted that odours produced an immediate effect on the central nervous system, releasing patients' memories and emotions, proving the effect of essential oils on psychology treatment (Agatonovic-Kustrin et al., 2020; Valnet, 2012). The essential oils are usually used to suppress bacteria (Karapinar & Aktuğ, 1987), to boost the spirit (Juergens et al., 2003), to soothe lungs (Xu & Huang, 2007; Fan et al., 2015) and for relax the mind (Javanmardi et al., 2002).

The name basil can trace its origin to the Greek basileus, which means 'king' (Singletary, 2018). The great English herbalist John Parkinson mentioned that the taste of basil is so good that it is very suitable for being used in the palace. The main herb of the bathing gel used by some ancient royal family in Italy is called 'the king of herbs' (Davis, 1995). Due to interspecific hybridisation and adaptation to the environment, various subspecies/varieties/ forms of basils exist and have similar appearance and chemotype. This hybridisation causes some problems in the classification of basil, as it is difficult to understand the heredity relation of basil (Grayer et al., 1996). Burzo and Mihaiescu (2005) show that different species and types have a different appearances and differ physiologically and chemically. Sweet basil, green basil, purple basil, and clove basil are entirely different in appearance and taxonomical classification, but the general public usually mix up these plants. Chuang (2008) used a molecular marker to decode the DNA of basils and pointed out that basils in Taiwan have considerable differences with species from overseas and speculates that it is caused by geographical isolation. Usually, basils are classified based on appearance or their chemical component. However, the planting environment and geographical conditions affect these two classification methods. Besides that, hybridisation has created significant confusion in the botanical systematics of the Ocimum genus (Vieira & Simon, 2006). Therefore, it is essential to consider the origin of the plant to overcome the problem of differences caused by the adaption ability of Lamiaceae.

The International Journal of Herbs in 2002 clearly defines the scientific name for sweet basil, green basil and purple basil. They are under the same family and genus (Mondello et al., 2002; Sajjadi, 2006). The general public tends to confuse the different basil plants mentioned, but they have different morphology. The leaves of sweet basil are oval and thicker when compared to green and purple basil. The green and purple basil leaves are sharper, thinner, and feel like paper. Green basil and purple basil have different stem colours, but green leaves. Clove basil, on the other hand, although from the same family Lamiaceae, is very different from that of sweet basil, green and purple basil. Clove basil and the other three types of basils have different applications. The previous basils are used in cooking because of their distinctive smell, while clove basil is usually used for liver maintenance in health care (Hung, 2006; Lin et al., 1993). In culinary, sweet basil is the one that is used to make Italian sauce, pesto. Sweet basil has a lighter flavour and is suitable with lighter flavour ingredients. If green or purple basil is used, it would taste like grass and has a much stronger taste (Hsu, 2013).

There are several commonly seen methods of extraction of essential oils, such as hydro-distillation (HD), solvent-free microwave extraction (SFME), microwave-assisted hydro-distillation (MAHD), steam distillation, solvent extraction, supercritical fluid extraction (Chenni et al., 2016; Da Silva et al., 2021; Tran et al., 2018). In the Early Middle Ages of Europe, towns mainly used the steam distillation method to produce perfumes, medical products, and alcohol, and advances in technology developed distillation equipment with different materials such as bronze and stainless steel (Kockmann, 2014). This method is currently widespread, and high-quality equipment can be easily obtained. Using the temperature and pressure of steam to destroy the oils capsules of the plant speeds up the release of essential oils by plants, both water-soluble and lipid-soluble components. In this way, two types of organic material can exist in the crude extract simultaneously to obtain complete essential oil extracts. The solvent extraction method uses an organic solvent to dissolve plant tissue. Many types of research on basil, sweet basil and clove basil also use this method (Ashish et al., 2021; Khater, 2020; Ciotea et al., 2021).

As extraction temperature cannot exceed 40°C, the essential oils are not affected by high temperature. It will not deteriorate quickly, but the polarity, boiling point and safety must be considered. Essential oils labelled 'absolute' in the market are produced by immersing plants in a volatile solvent such as petroleum ether or hexane to shift the aroma ingredient (Yih et al., 2014). However, this method is susceptible to solvent residue and might affect the extraction quality. If there are no specific needs, this method is not commonly used in the market and research, and this method also cannot obtain herbal distillate, and cannot obtain a total crude extract, thus does not meet the requirement of this research. The supercritical fluid extraction (SFE) method uses carbon dioxide as a medium of extraction, adjusting the temperature and pressure to a supercritical condition. The supercritical fluid phase is between the gas phase and liquid phase, the viscosity of the fluid is nearer to that of gas and its density nearer to that of liquid; the essential oils are dissolved out of the plant under this high pressure. The SFE with carbon dioxide method takes a shorter time, and there is no chemical reaction between solvent and essential oils. The whole process is carried out at a low temperature and is suitable for essential oils components that are unstable at high temperatures. However, the disadvantage is the need for large equipment and high cost, and this is only used to extract precious essential oils (Yih et al., 2014; Arranz et al., 2015). This method cannot harvest liquid crude extract; it cannot obtain the water-soluble component, thus unable to analyse the fundamental elements. Comparing the mentioned methods, solvent extraction and supercritical fluid extraction method can only extract lipid-soluble essential oils components, but not water-soluble components. The solvent extraction method might have an incomplete solvent reaction, and solvent residue in the oil extract, plus these two methods easily cause incomplete oil extract. Therefore, steam distillation, the commonly used technique to harvest complete crude extract, is used. This distillation ensures that the complete lipid-soluble and water-soluble essential oil component is obtained (Arumugham et al., 2021).

This study aims to investigate the components of four types of basils commonly found in Taiwan: Ocimum basilicum L. widely known as sweet basil; Ocimum basilicum L. cv. green known as green basil; Ocimum basilicum L. var. purpurascens known as purple basil; and Ocimum gratissimum L. known as clove basil. The general public usually cannot tell the differences between these basils and consider them the same species. However, they are different, and so are their essential oils (Hussain et al., 2008; Patel et al., 2016). For this study, the plants are carefully selected, and their flowers and leaves are carefully harvested to be analysed by gas chromatography-mass spectrometry (GC-MS). Furthermore, the essential oils of the four types of plants are evaluated by experienced aromatherapists through a questionnaire of closed-ended questions. The questionnaire is designed based on the results of GC-MS. This study aims to describe and give a further understanding of the chemical content of basils and hopes to guide the use of basils in aromatherapy. The structure of the paper is as follows. In Section 2, we present the materials and methods. Then, in Section 3, the results and discussions. Finally, conclusions are drawn in Section 4.

2. MATERIALS AND METHODS

Before the experiment, a pre-experiment is carried out to observe and record distillation time, heating temperature, and the changes in the amount of extract. The plant used in pre-experiment is purchased readily for distillation. The essential oils obtained are analysed using GC-MS (Adams, 2007; Muráriková et al., 2017; Sacchetti et al., 2005). The distillation time to get the extract for each plant is obtained, and the extract is analysed. After the analysis, the optimum condition for extraction and the hypotheses of aroma evaluation is proposed. Based on the observed optimum condition in the pre-experiment, a second distillation is carried out using plants from the exact origin, and the extract is analysed using GC-MS. Finally, a questionnaire is designed using the proposed hypotheses and evaluated by experienced aromatherapists to differentiate the four types of basils.

2.1 Plant material for pre-experiment

Before the distillation, four types of readily available plants are purchased to carry out the pre-experiment to define the optimum distillation time and heating temperature. The purchase of the plants was made in the following places: for sweet basil in Changhwa Fanwa Farm (address: No.185, Douyuan Road, Ren'ai Village, Fangyuan Township, Changhua County); for green basil in Miaoli's herbs planting farmer; for purple basil and clove basil in Jinfutian Organic Farm (address: No.2, Lane 795, Dongkeng Road, Dongshi District, Taichung City). The harvesting part used for distillation is the flowers and leaves with two kilograms for each plant. First, dust and dirt are washed off the gathered flowers and leaves. Then they are dried in the shade for 12 hours to remove surface water.

2.2 Plant material for the experiment

Four types of plants planted in the same place are extracted under the optimum condition in the experiment. The source of seeds and plants are from merchants who previously worked with research units or experts, to make sure the herbs are of the right species. Organic farms planting herbs provide three types of plants in seeds, and one type of plant in angiosperms, to make sure the origin of the species are correct. The sweet basil angiosperm was obtained from Tangshan Herb (address: No. 36, Chungwen 3rd Street, Puli Township, Nantou County). The clove and purple basil seeds were obtained from

Jinfutian Organic Farm (address: No.2, Alley 795, Dongkeng Rd., Dongshi Dist., Taichung City). The seeds of green basil were obtained from House of Vegetables (address: No.233-1, Section 2, Hecuo Road, Hemei Township, Changhua County). Then, the four types of plants are planted in Tainan City in Taiwan for three months. The number of sweet basil's angiosperms is increased by cutting. Approximately 10-15 cm of the stems are cut and are placed in a bottle filled with tap water. After nearly ten days, the grown roots are moved to small pots containing soil. The plant is sufficiently watered every day. Plants are transferred to larger containers when grown to approximately 15 centimetres tall. Sweet basil, green, purple, and clove basil take around 1 to 1.5 months to blossom. Then, the flowers and leaves are harvested for distillation. The plants were harvested, ensuring that the previous ten-day had not rained to avoid the essential oils being flushed away by rain. The flowers and leaves collected for the experiment are directly distilled without washing and drying. The plants are extracted on the same day they are harvested.

2.3 Extraction method

As essential oils contain both water-soluble and lipid-soluble components, it is crucial to retain the original elements as possible. Therefore, a pre-experiment is carried out to determine the relationship between distillation time and heating temperature and the content of the extract. The result of the pre-experiment is used to determine the optimum distillation time and heating temperature for extraction. At the same time, the result of the pre-experiment is also used to propose five hypotheses for the aroma evaluation. This research uses distillation equipment commonly found in the market. The Taishan's TS003-10L, all 304 stainless steel material made in Taiwan, is used. Two kilograms of freshly harvested flowers and leaves are weighed and distillate in pre-experiment, while three kilograms are used in the experiment, corresponding to the second distillation. Each distillation lasts for three hours in the pre-experiment, and the essential oils are collected every hour. The amount of essential oils is observed to decide the optimum distillation condition for the experiment. After pre-experiment, a second distillation is carried out using the optimum condition based on observations of the pre-experiment. Both distillations are in pre-experiment, and the experiment is carried out using the same equipment. The same method is used to extract essential oils in both the first and second distillation. The distiller is sterilised before distillation. An induction stove is prepared—the duration and temperature of the distillation set. Five litres of distilled water is added to the distiller. Flowers and leaves are weighed and placed on the separation rack, and the distiller is covered with its lid. The separation rack separates the flowers and leaves from the distilled water to be heated uniformly. For the first thirty minutes, the distiller is heated to a temperature of two hundred Celsius (200°C) and maintained at that temperature. After that, the essential oils start to flow out, and the temperature is maintained at a lower temperature to prevent overheating. The flowers and leaves are heated for three hours in the pre-experiment, and their essential oils are collected every hour.

2.4 Gas chromatography-mass spectrometry analysis

The collected essential oils are diluted 1000 times with ethyl acetate (EA). Then, it is analysed using gas chromatography-mass spectrometry (GC-MS). GC-MS is carried out using Shimadzu's GC-2010 with a GCMS-QP2010 detector and an AOC-20i injector, fitted with a GL Sciences' InertCap-5MS/NP column (0.25 mm I.D. x 30 m, df = 0.25 µm). 1.5 mL of the diluted essential oils are filled into sample bottles. They are placed accordingly onto a rotating tray for elution. The pressure of the tank is set between 0.7-0.8 MPa. Then, the GC-MS and computer are switched on accordingly. The results obtained from GC-MS analysis are examined and compared with the ISO Standards.

2.5 Aromatherapy evaluation

According to the data obtained from GC-MS analysis in the pre-experiment, five hypotheses are proposed to construct the essential oils aroma evaluation form filled by experienced aromatherapists. Thirty female aromatherapists, aged between 26 and 30, with more than four years of experience in aromatherapy, are invited to evaluate the aroma of the essential oils extracted. The essential oils are filled into labelled spray bottles. The bottles are shaken before spraying, and they are sprayed at an angle of 45° and a distance of 30 centimetres away from the face of the aromatherapist. In between each spraying, aromatherapists inhale coffee beans to clear the scent left in the nostril and breath, usually for 20 seconds. All aromatherapists then mark every five hypotheses in the evaluation form based on the aroma of the essential oils and their feeling after inhaling. Each hypothesis is rated on a numeric scale of 1 through 5, with one being strongly disagreed and five strongly agree. Thus, with 30 aromatherapists, each hypothesis has the highest score of 150, which is also a perfect score. The ratings given by all 30 aromatherapists are summed to obtain a single actual score. The supporting ratio is obtained by dividing the actual score by the highest score.

3. RESULTS AND DISCUSSION

Pre-experiment is carried out using two kilograms of flowers and leaves in five litres of distilled water to observe the relationship between duration of distillation, heating temperature, and amount of crude extract harvested. The pre-experiment shows that the optimum time and temperature for extraction are increased to 200°C for the first 30 minutes and decreased to and controlled at 160°C for the next 30 minutes. The plants turn brown after heating. At this condition, the essential oils are maintained at a constant flow. This prevents overheating and the distilled water from evaporating too quickly. However, the high temperature will destroy the components of essential oils. If it is heated at a temperature lower than 160°C, the flow of essential oils is obstructed, and the plants remain freshly green after extraction, which means the essential oils are not thoroughly extracted. Table 1 and Table 2 show that extract collected after 2 and 3 hours of distillation for sweet basil shows incomplete components. This also means that most elements can be obtained with a distillation of 1 hour. For green basil, it is found that although the amount of methyl chavicol increases with distillation time, the loss of components also increases. The main components of purple basil increase with distillation time; however, the content of other elements decreases

As for clove basil, its main components start to slip away after 1 hour. The yield for purple basil and clove basil is highest within 2 hours, while sweet basil and green basil have the highest return within 1 hour. All four types of basils are completely extracted within 2 hours through the steam distillation method. As the aim of distillation in this research is to obtain essential oils in their most complete form possible, it is decided that 1 hour is the distillation time for all basils in this research.

Weight of flowers and leaves (kg)	Distilled water (L)	Temperature condition	Amount of extract (L)			
			Sweet basil	Green basil	Purple basil	Clove basil
2	5	200°C throughout	3.5	3.7	3.5	3.6
2	5	First 30 minutes: 200°C Last 30 minutes: 180°C	2.6	2.5	2.4	2.6
2	5	First 30 minutes: 200°C Last 30 minutes: 160°C	1.5	1.5	1.5	1.5

Table 1. Temperature condition and extract collected after 3 hours in the pre-experiment

Compound	Molecular weight (g/mol)	Hour	Composition (%)			
			Sweet basil	Green basil	Purple basil	Clove basil
Eucalyptol ($C_{10}H_{18}O$)	154.25	First	7.69	1.01		
		Second	0.59	0.06		
		Third				
Linalool ($C_{10}H_{18}O$)	154.25	First	39.54			
		Second	1.24			
		Third				
Eugenol ($C_{10}H_{12}O_2$)	164.204	First	47.89	2.29		86.05
		Second	1.02	1.01		73.58
		Third				63.98
Methyl chavicol ($C_{10}H_{12}O$)	148.2	First		96.70	95.95	
		Second		98.30	97.23	
		Third			100.00	
β-Ocimene ($C_{10}H_{16}$)	136.23	First			2.05	
		Second			0.00	
		Third			0.00	
α-Bergamotene ($C_{15}H_{24}$)	204.35	First			2.00	
		Second			2.77	
		Third			0.00	
cis-Ocimene ($C_{10}H_{16}$)	136.24	First				3.56
		Second				3.42
		Third				0.00
Copaene ($C_{15}H_{24}$)	204.36	First				0.00
		Second				4.46
		Third				4.26
B-Caryophyllene ($C_{15}H_{24}$)	204.36	First				3.25
		Second				4.64
		Third				6.71
Germacrene D ($C_{15}H_{24}$)	204.35	First				7.14
		Second				13.90
		Third				22.85
δ-Cadinene ($C_{15}H_{24}$)	204.35	First				0.00
		Second				0.00
		Third				2.20

Table 2. Results of GC-MS analysis in the pre-experiment

Table 3 shows the results obtained by steam distillation of the four types of basils at the conditions determined from the pre-experiment, heating to 200°C for the first 30 minutes and maintaining at 160°C for the next 30 minutes. Results of GC-MS analysis are shown in Table 4, and the mass spectrum of sweet basil, green basil, purple basil and clove basil are shown in Figures 1 to 4, respectively.

Weight of flowers and leaves (kg)	Distilled water (L)	Temperature	Amount of extract after 1 hour (L)			
			Sweet basil	Green basil	Purple basil	Clove basil
3	5	First 30 min: 200°C Last 30 min: 160°C	0.5	0.5	0.5	0.5

Table 3. Extract collected from sweet basil, green, purple and clove basil after 1 hour in the second distillation

Compound name	Composition (%)			
	Sweet basil	Green basil	Purple basil	Clove basil
Eucalyptol	8.86	2.73		
Linalool	40.01		0.70	
Methyl chavicol	0.21	93.72	94.32	
Camphor	0.25			
Bornyl alcohol	0.93			
α-Terpineol	1.84			
Eugenol	46.72	3.08		83.41
α-Bergamotene	0.60	0.47	2.13	
Muurolol	0.58			
β-Ocimene			2.85	
cis-Ocimene				6.31
β-Caryophyllene				2.66
Germacrene D				7.62

Table 4. Results of GC-MS showing percentage composition of essential oils in the second distillation

Sweet basil's essential oils are mainly eucalyptol, linalool, and eugenol, while other trace components such as camphor and muurolol help regulate its aroma. Trace component α-bergamotene gives sweet basil extra orangey scent and works well with the main component eucalyptol to offers a fresh, energetic blend. From GC-MS data, it is known that the component of sweet basil is very diverse. It is composed of many trace components that coordinate the overall aroma and is also widely accepted. Green basil's main component, methyl chavicol, constitutes more than 93% of its essential oils. The scent of trace component bergamotene eases the irritation brought by the main component. The main elements of sweet basil, eucalyptol, and eugenol, are also found in green basil, with a difference in content. Both these ingredients increase the fresh and lively aroma of green basil. Purple basil has the same main component as green basil. Methyl chavicol constitutes more than 94% of its essential oils. The same trace component bergamotene is also found in purple basil, which slows down the irritation brought by the main component. The other component linalool gives purple basil's essential oils a sense of calmness. Results show that clove basil has different elements from the other three basils. Although it has the same main component eugenol, as sweet basil, its amount is much higher than that in sweet basil, other trace components found in clove basil combined as the ingredient which soothes the lungs.

From Figure 5, it is confirmed that the main components of green basil and purple basil are almost the same. While green basil and sweet basil have a lot of shared components, they are very different in amount, which means there is little correlation between the two. Clove basil and sweet basil have only one component in common. Other components found in sweet basil are

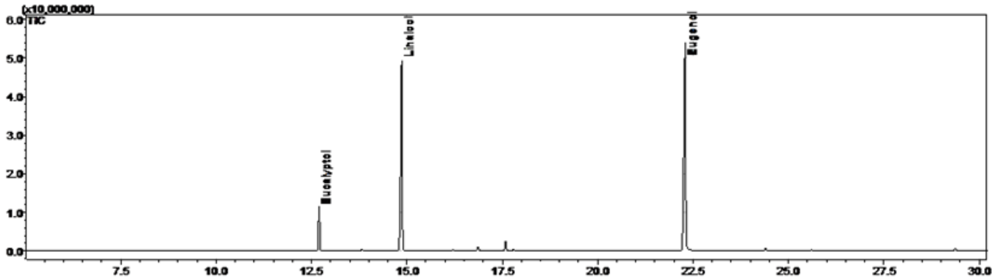

Figure 1. Mass spectrum of sweet basil

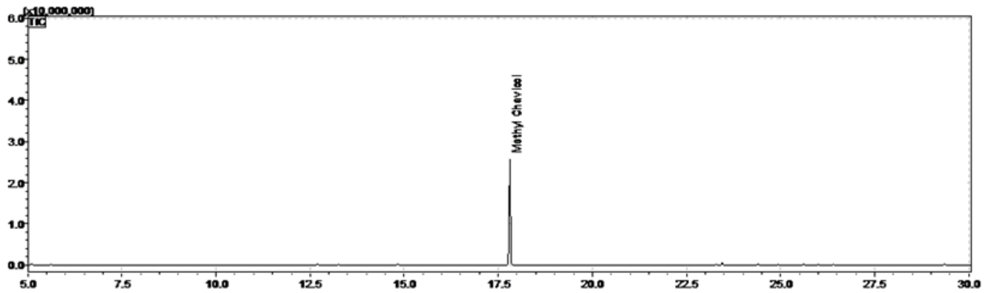

Figure 2. Mass spectrum of green basil

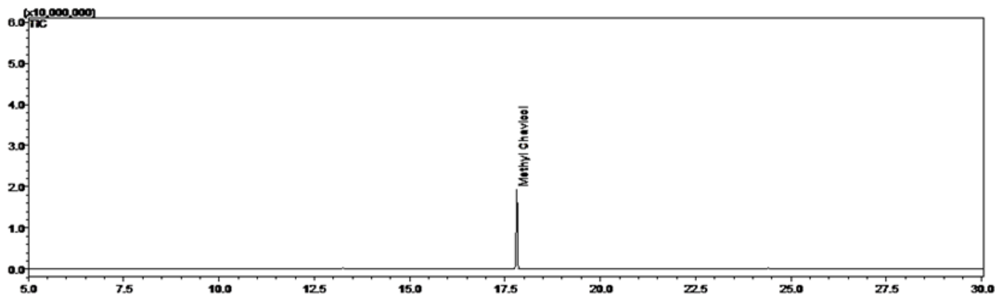

Figure 3. Mass spectrum of purple basil

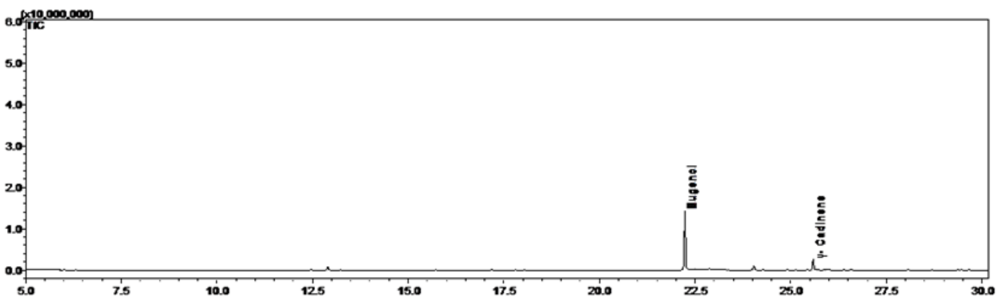

Figure 4. Mass spectrum of clove basil

not found in clove basil. From the perspective of the analysis of the components of essential oils, it is shown that green and purple basil, sweet basil and clove basil are different type of plants. Although the retention times of eugenol of different basil are slightly different, from Figure 6, it is clearly shown that both eugenols found in sweet basil and clove basil are the same. Table 5 shows a vast difference in the content of green and purple basil with sweet basil and clove basil, which further indicates that these basils are different from the components of their essential oils.

Main component	Retention time (s)	Content (%)			
		Sweet basil	Green basil	Purple basil	Clove basil
Eucalyptol	12.70	8.68	2.73	0.00	0.00
Linalool	14.86	40.10	0.00	0.70	0.00
Methyl chavicol	17.80	0.21	93.72	94.32	0.00
Eugenol	22.29	46.72	3.08	0.00	83.41

Table 5. The content of principal components in four types of basils

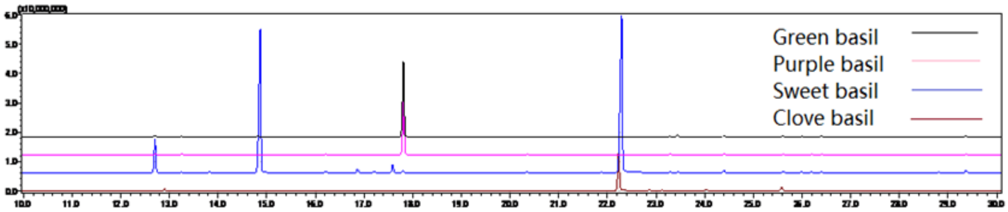

Figure 5. Comparison of GC-MS analysis of essential oils of four types of basil

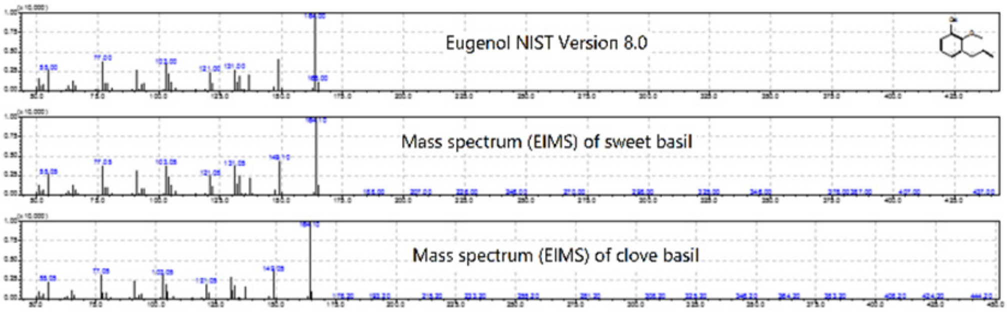

Figure 6. Mass spectrum of eugenol in sweet basil and clove basil

From Figure 5, it is confirmed that the main components of green basil and purple basil are almost the same. While green basil and sweet basil have a lot of shared components, they are very different in amount, which means there is little correlation between the two. Clove basil and sweet basil have only one component in common. Other components found in sweet basil are not found in clove basil. From the perspective of the analysis of the components of essential oils, it is shown that green and purple basil, sweet basil and clove basil are different type of plants. Although the retention times of eugenol of different basil are slightly different, from Figure 6, it is clearly shown that both eugenols found in sweet basil and clove basil are the same. Table 5 shows a vast difference in the content of green and purple basil with sweet basil and clove basil, which further indicates that these basils are different from the components of their essential oils.

Table 6 shows the comparison of GC-MS results with ISO Standard. It is observed that the components that appeared in the analysis results are also found in the ISO Standard (International Organization for Standardization [ISO], 1998). This reflects that the source of plants, extraction method and analysis method in this research are reliable, and the results are credible and can be used as a reference for aromatherapy and other related applications. Results of GC-MS analysis show that the main components of the essential oils of sweet basil, green basil, purple basil and clove basil are different. The main component of green basil and purple basil's essential oils is methyl chavicol, which constitutes 93.72% and 94.32%, respectively. Mass spectrometry shows that the retention time for both the main components of green basil and pur

Component	Minimun (%)*	Maximum (%)*	Content (%) Sweet basil	Green basil	Purple basil	Clove basil
Eucalyptol (1,8-cineole)*	1.00	3.50	8.68	2.73		
trans-Ocimene*	0.90	2.80			2.85	6.31
Camphor*	0.15	0.80	0.25			
Linalool*	0.50	3.00	40.10		2.13	
Terpinen-4-ol*	0.20	0.60	1.84			
Methyl chavicol*	75.00	87.00	0.21	93.72	94.32	
Eugenol*	0.30	2.50	46.72	3.08		83.41
Bornyl alcohol			0.93			
α-Bergamotene			0.60	0.47	0.70	
Muurolol			0.58			
β-Caryophyllene						2.66
Germacrene D						7.62

*NOTE: From chromatographic profile ISO 11043:1998(E). The chromatographic profile is normative, contrary to typical chromatograms given for information.

Table 6. Comparison of GC-MS results with ISO Standard

ple basil are the same, which is 17.80 seconds, and they are the same compound. Moreover, both sweet basil and green basil have eucalyptol and eugenol, but green basil has a lower content. There are three main components in sweet basil: eucalyptol with a retention time of 12.70 seconds constitutes 8.68%, linalool with a retention time of 14.86 seconds constitutes 40.10%, and eugenol with a retention time of 22.29 seconds represents 46.72%. Sweet basil also contains a trace amount of methyl chavicol, at 0.21%. The main component of clove basil's essential oils is eugenol, which takes up 83.41% of all compounds, with a retention time of 22.29 seconds. Besides that, clove basil has a higher eugenol content than sweet basil.

Summarising the uses of the four types of basil and the results of GC-MS, five hypotheses for aroma evaluation by aromatherapists can be proposed.

- Hypothesis A: Since there is no distinction between green and purple basil in cooking, green basil and purple basil are considered the same plant and have the same components.
- Hypothesis B: If green and purple basil are categorised as the same plant, and sweet basil and clove basil are used differently, green basil and purple basil are considered a different plant with sweet basil and clove basil according to their aroma.
- Hypothesis C: As pesto made from sweet basil is regarded with great favour by the general public;

thus, the aroma of sweet basil extract is widely favoured by the general public.

- Hypothesis D: There is much literature related to the antimicrobial property, lungs soothing effect, and immunity-boosting properties of clove basil in the Asia region; therefore, aromatherapists feel comfortable in their lungs after inhaling clove basil's essential oil.
- Hypothesis E: Since the scent of sweet basil is favoured by the general public, then the component of sweet basil's essential oil gave a feeling of happiness and relaxation after inhaling.

Thirty questionnaires were distributed and collected. The questionnaire is shown in Appendix A. Results in Table 7 support these research hypotheses positively. Among 30 aromatherapists, 26 support all five hypotheses in this evaluation. Hypotheses A and B received the highest supporting ratio, with 90% and above acceptance. 29 out of 30 aromatherapists prefer the aroma of sweet basils among all four essential oils. Only one aromatherapist does not particularly favour its aroma. Twenty-eight aromatherapists find that inhaling clove basil's essential oils soothes their lungs, and 29 aromatherapists find that inhaling sweet basil's essential oils gives a sense of happiness and relaxation. This aroma evaluation shows that more than 90% of all the aromatherapists in this evaluation think that green basil and purple basil have the same essential oils components. Higher content of methyl chavicol is not favoured in the aroma evaluation. Most aromatherapists decide that sweet basil, green and purple basil, and clove basil are different plants with different essential oils components. Components of sweet basil are more diverse, have higher support, and are highly favoured by the general public. Most aromatherapists also agree that the aroma of sweet basil helps relax the body and mind. Aromatherapists also think that inhaling clove basil's essential oils can soothe the lungs.

Hypothesis	Scale					Supporting ratio
	1	2	3	4	5	
A	0	0	0	15	15	0.90
B	0	0	0	12	18	0.92
C	0	0	1	18	11	0.87
D	0	0	2	13	15	0.89
E	0	0	1	16	13	0.88

Table 7. Results of aroma evaluation

4. CONCLUSIONS

From the chemical components of essential oils of the four types of basil analysed, it is found that they have similar chemical elements and similar content compared to ISO 11043:1998(E) Standards. Many kinds of research state that clove basil is a valuable plant, and it is confirmed that its essential oils contain high levels of eugenol (83.41%), and it is used to soothe the lungs. Further research should clarify the properties of the plant and its medicinal value. Analysis data shows that green basil and sweet basil share some components such as eucalyptol, methyl chavicol, eugenol and α-bergamotene. It is suggested that they are related, and further research is required to find out more evidence about them. Green basil and purple basil can be considered the same plant from their essential oils because they share a high amount of methyl chavicol (93.72% and 94.32%, respectively). Sweet basil, green, purple, and clove basil are easily confused by the general public. This research confirmed that these basils appear differently in the aroma of their essential oils. The component of sweet basil is more diverse than that of the other basils. Its aroma is also relatively gentler and less pungent; therefore, it is generally more acceptable and commonly used in cooking and aromatherapy. Aromatherapists confirm that clove basil has a lung-soothing effect, and it is suggested to be added in lung-soothing products such as incense and oils. Aromatherapists also consistently agree that the aroma of sweet basil relaxes the mind and body. It can bring relaxation and a calm to anxious mood when facing tense situations, thus helping stabilise performance.

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