Microscopic and Chemical Composition Study of the Essential Oils from Turmeric Rhizome and Holy Basil and Kaffir Lime Leaves Grown in Thailand.

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Abstract

This study aims to identify the medicinal properties of the active compounds of three Thai herbs: *Curcuma longa* L. (turmeric) and *Citrus hystrix* DC. (kaffir lime), and *Ocimum tenuiflorum* L. (holy basil), with a focus on their essential oils. These herbs are renowned for their high concentrations of essential oils known for their aromatic and pharmacological properties. Utilizing plant material collected from central Thailand, the study conducted a comprehensive investigation involving microscopic study, essential oil extraction, and GC-MS analysis. Employing the free-hand sectioning technique and examining samples under a light microscope yielded the following observations: oil droplets were detected within the parenchyma cells of turmeric, oil glands were observed in the glandular trichomes of holy basil, and oil glands were identified in the epidermal cells of kaffir lime. Our findings revealed distinct anatomical features and chemical compositions of the herbs' essential oils. Turmeric exhibited compounds with potential anti-ulcer properties, with the main constituents being ar-turmerone (47.39%), curlone (17.75%) and turmerone (14.44%). Kaffir lime contained constituents with antimicrobial activity, predominantly 6-Octenal, 3,7-dimethyl-, (R)- (85.56%), and citronellol (7.39%). Holy basil showcased antioxidant properties, with caryophyllene (69.98%) and eugenol (12.98%) being the major constituents. However, safety concerns are associated with herbal medicine usage, especially without understanding its chemical constituents. By identifying key compounds that have been reported to have medicinal properties, this research contributes to a deeper understanding of herbal remedies and their potential role in enhancing public health outcomes. Further research is needed to explore these herbal medicines' therapeutic efficacy and safety profiles.

Keywords: *Essential Oils, Holy Basil, Turmeric, Kaffir Lime, Chemical Composition, Herbal Medicine*

1. Introduction

Herbal medicines have been widely used in developed and developing countries, particularly in regions with limited access to modern healthcare. Martins Ekor (2013) showed that approximately 80% of the global population relies on herbal medicines as a primary source of healthcare; exploring their potential effectiveness becomes imperative (Ekor, 2013). Herbal medicine is a field yet to be explored and discovered, containing many beneficial pharmacological properties and therapeutic efficacy of herbal medicine. Various studies underscore the potential therapeutic advantages of herbal medicine and remedies. The herbs utilized in this project are *Curcuma longa* L. (turmeric), *Citrus hystrix* DC. (kaffir lime), and *Ocimum tenuiflorum* L. (holy basil). These herbs are prevalent in Thailand, underscoring the need to investigate their properties. Given their frequent incorporation into Thai cuisine, these herbs are already part of the dietary intake of individuals in Thailand. The study will also consider the ratios of oil glands to biomass, providing additional insights into the factors influencing oil yield variations among the studied plants. Shivkanya Fuloria et al. (2022), they have conducted a literature review alongside exploring the use of nanotechnology to treat wounds for *Curcuma longa* L. in India. Fuloria and her colleagues found that *Curcuma longa* L. had active compounds ar-turmerone (40%), α-turmerone (10%), and curlone (23%), and had therapeutic properties such as its anticancer and antioxidant activities (Fuloria et al., 2022). Padma Vasudevan *et al.* (1999) extracted the essential oil from *Ocimum tenuiflorum* L. and identified the active compounds and properties of the herb. The study identified active compounds such as eugenol (57-50%), caryophyllene (22-32%), and methyl eugenol (6-14%), and their anti-inflammatory effects (Vasudevan et al., 1999). Furthermore, a systematic review by Hawa Nordin Siti et al. (2022) explored the diverse potential benefits of *Citrus hystrix* DC., revealing its efficacy in treating stomach aches, diabetes, hypertension, and diarrhea, among many other conditions. While the active compounds were identified, the specific percentages in the essential oil were not stated (Siti et al., 2022). By contrasting and comparing the active compounds present in each herb, a deeper understanding can be gained of how different environments, such as those in India and Thailand, may affect the herbs. Alongside the therapeutic properties, understanding the herbs could benefit the economy, turning the raw ingredients into profitable products. This project is aligned with Thailand's Sustainable Development Goals (SDGs), particularly the third, eighth, and twelfth goals. The third goal emphasizes the well-being of individuals, highlighting the significance of understanding herbs and maximizing their benefits for fostering a healthier ecosystem. Moreover, the eighth goal is addressed as this project fosters economic growth through collaboration between local farmers and medical experts, thereby advancing the herbal medicine field. Lastly, the twelfth goal concerning responsible consumption and production is addressed through the utilization of local herbs in this project. This paper aims to explore three herbs' key chemical bioactive compounds and assess their potential therapeutic effects.

2. Objectives

This project seeks to elucidate the properties of *Curcuma longa* L., *Citrus hystrix* DC., and *Ocimum tenuiflorum* L., three herbs renowned for their therapeutic potential. In essence, this paper aims to explore the key chemical bioactive compounds present in three herbs and assess their potential therapeutic effects.

3. Materials and Methods

3.1 Plant Material

The herbal raw materials utilized in this study comprised *Curcuma longa* L. (turmeric) rhizome and *Citrus hystrix* DC. (kaffir lime) leaves, and *Ocimum tenuiflorum* L. (holy basil) leaves. These herbs are known for their essential oils and were collected in the central region of Thailand. These herbs were identified by Nirun Vipunngeun, a botanist lecturer in the Department of Pharmacognosy, College of Pharmacy, Rangsit University. The herbs are referenced by their corresponding herbarium collections at BKF. *Curcuma longa* L. is referenced by the botanical number 143148, *Citrus hystrix* DC. by 086254, and *Ocimum tenuiflorum* L. by 197895. The herbs underwent a cleansing process with clean water, followed by drying and later being segmented into smaller pieces.

3.2 Microscopic Study

The herbal parts, such as the leaves and the rhizome, were cut using a razor blade into thin slices using a free hand section. These sections were then observed under a light microscope (BA300; Motic, Xiamen, China).

3.3 Extraction

Water distillation was employed to extract the essential oils from the respective herbs, with samples obtained from holy basil leaves, turmeric rhizome, and kaffir lime leaves. These plant components were finely chopped and placed in round-bottomed flasks containing 2 liters of water, where the water served as the condenser. The herbs were filled to approximately half of the round-bottomed flask's volume. The graduated tubes were filled with water according to their standardized line. The heating process was initiated, and distillation proceeded for 5 hours. The liquid within the flask reached temperatures of 130°C - 150°C, leading to the evaporation of water from the herbs. Subsequently, the essential oil exited through steam into the graduated tube. The essential oils, coexisting with water, underwent a separation based on density, allowing the extraction of the essential oil into a distinctive bottle. The collected essential oils are then quantified and used to determine the yield percentage through the division of the essential oil quantity by the initial weight of the respective herbs, concluding the essential oil extraction.

3.4 Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The chemical composition of the essential oils and powders of the respective herbs is then analyzed by the Gas Chromatography-Mass Spectrometry (GC-MS), specifically the model GC 7890 A. The column employed was of type DB-5MS, with dimensions of 30m x 0.25 mm, a diameter of 0.25 mm, and a film thickness of 0.25 microns. The carrier gas utilized was helium at a 1 ml/min flow rate. A 1-microliter sample volume was injected, employing a split mode at a ratio of 10:1. The sample chamber was maintained at a temperature of 270°C. The incubator temperature was initiated at 50°C and increased at a rate of 3°C per minute until reaching 240°C. Subsequently, the temperature was elevated at a rate of 5°C per minute until a final temperature of 270°C was sustained for 5 minutes. The analysis mass range spanned from 40 to 900 amu, with a total analysis time of 70 minutes post-separation. Chromatograms and spectra were collected, providing a basis for comparative essential oil analysis. The mass spectra of individual substances separated into essential oils were compared with the mass spectra of various standard substances. The data was represented as the mean percentage of three trials of this experiment, with a low standard deviation to show the consistency of the active compounds.

4. Results and Discussion

4.1 Macroscopic properties

The rhizome of *Curcuma longa* L. (Figure 1 - A), has an average diameter of 1.7 cm. The leaves of *Citrus hystrix* DC. (Figure 1 - B), were, on average, approximately 2.5 cm wide and 8.5 cm tall. Lastly, the leaves of *Ocimum tenuiflorum* L. (Figure 1 - C) were, on average, approximately 3 cm wide and 6 cm tall.

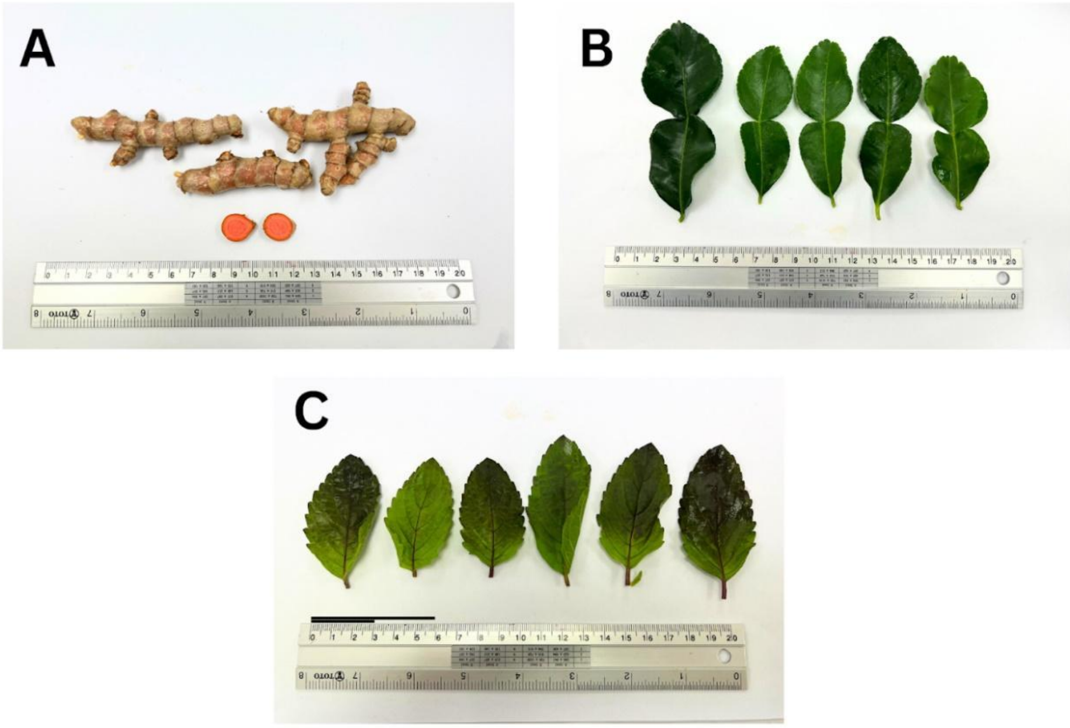

Figure 1: *Curcuma longa* L., *Citrus hystrix* DC., and *Ocimum tenuiflorum* L. were utilized in this study.

4.2 Microscopic properties

Plant oil glands are structures that produce and store essential oils and are used for various purposes, such as defense against herbivores, attraction of pollinators, and protection against pathogens. Turmeric oil droplets in *Curcuma longa* L. (Figure 2 - A) are typically observed in the rhizomes, which are the underground stems of the plant. These oil droplets appear small, spherical, or oval-shaped, located inside the parenchyma cells of the rhizome tissues. The droplets contain essential oils surrounded by specialized cells. Under a microscope, these droplets may exhibit a translucent appearance due to the accumulation of essential oils within them. *Ocimum tenuiflorum* L. (Figure 2 - B), possesses oil glands primarily in its leaves and flowers. These glands are observed as small, spherical structures located within the epidermal cells of the leaf and flower tissues. These glands are located within glandular trichomes found in the epidermis of *Ocimum tenuiflorum* L., particularly prevalent in plants of the Lamiaceae family. Under a microscope, the oil glands of holy basil may appear densely packed within the leaf and flower tissues, contributing to the characteristic aroma of the plant. In *Citrus hystrix* DC. (Figure 2 - C), oil glands are primarily found in the fruit's peel or zest and in the leaves' pockets. These oil glands appear as specialized structures within the fruit peel's exocarp and leaf tissues' mesophyll. The glands consist of secretory cells, or idioblasts, that accumulate essential oils, giving them a distinctive appearance under the microscope. Depending on their location within the plant tissue, these glands may exhibit varying shapes, such as spherical or elongated.

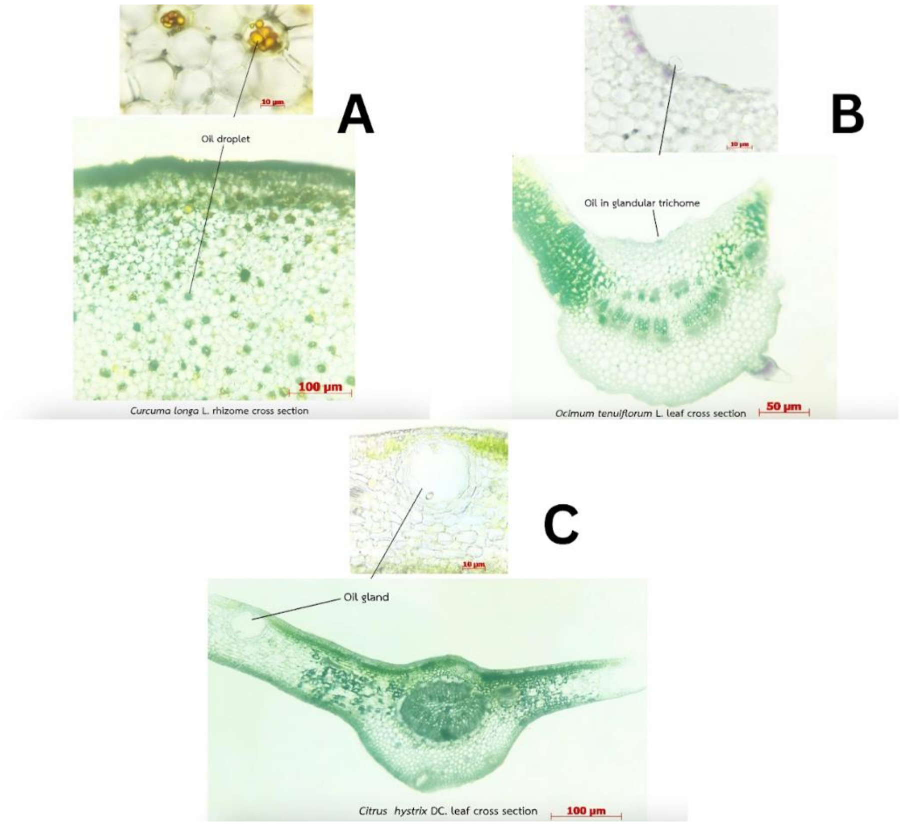

Figure 2: Anatomical characteristics of the transverse sections of the rhizome of turmeric, holy basil leaf, and kaffir lime leaf, respectively.

Table 1: Essential oils yielded from each herb

Herbs	Grams	Oil Yielded	Yield Percentage
Ocimum tenuiflorum L. (leaves)	300	0.4 ml	0.13%
Curcuma longa L. (rhizome)	800	1.5 ml	0.1875%
Citrus hystrix DC. (leaves)	129	1.2 ml	0.93%

The oil volume from 300 grams of *Ocimum tenuiflorum* L. leaves was 0.4 milliliters of essential oil, making an oil yield percentage of 0.13%. This was followed by 800 grams of *Curcuma longa* L. rhizome, which yielded 1.5 milliliters of essential oil, resulting in an oil yield percentage of 0.1875%. Lastly, 129 grams of *Citrus hystrix* DC. Leaves yielded 1.2 milliliters of essential oil, with an average yield of 0.93%.

4.4 Gas Chromatography-Mass Spectrometry Analysis of the Essential Oils from Turmeric Rhizome, Holy Basil, and Kaffir Lime Leaves

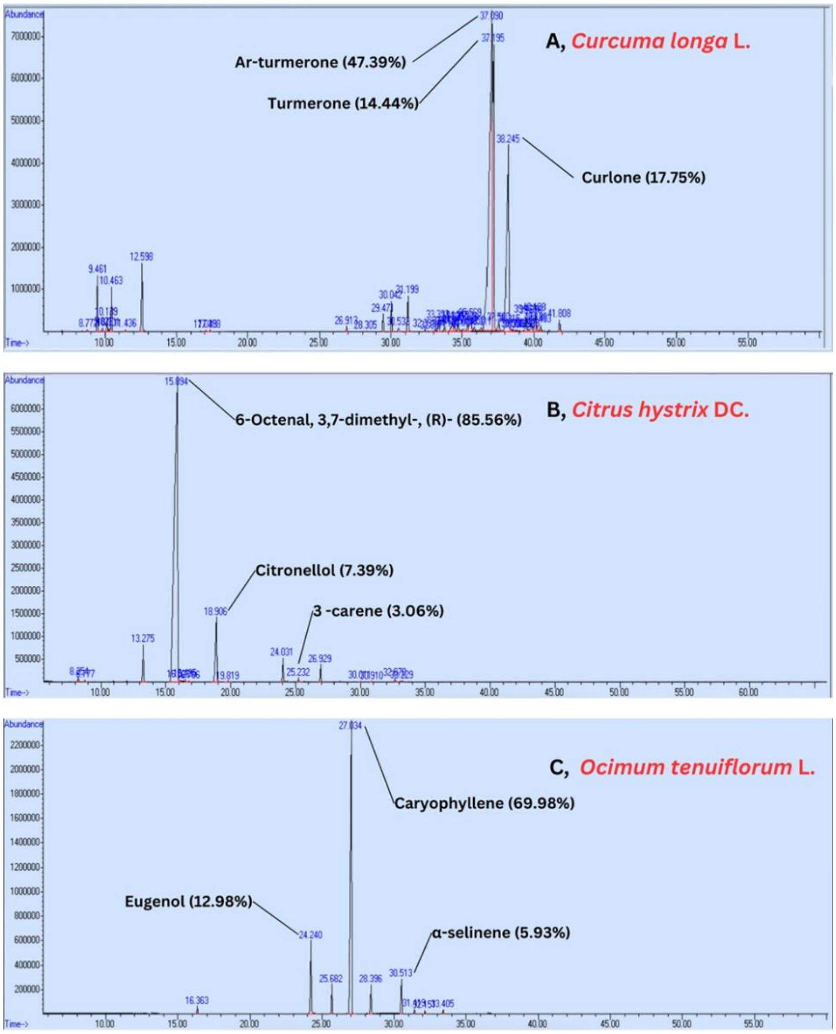

Figure 4: GC-MS graphs for the active compounds of *Curcuma longa* L. (A), *Citrus hystrix* DC. (B), and *Ocimum tenuiflorum* L. (C), respectively.

The analysis of the oil composition extracted from turmeric rhizome (*Curcuma longa* L.), labeled A in Figure 4, using the water distillation method revealed the presence of ar-turmerone (47.39%), curlone (17.75%), turmerone (14.44%), and other compounds. Moreover, the analysis of kaffir lime leaves (*Citrus hystrix* DC.), labeled B on Figure 4, oil composition obtained through water distillation identified 6-Octenal, 3,7-dimethyl-, (R)- (85.56%), citronellol (7.39%), 3 -carene (3.06%), and other constituents. Furthermore, the analysis of holy basil leaves' (*Ocimum tenuiflorum* L.), labeled C in Figure 4, oil composition, also extracted through water distillation, revealed the presence of caryophyllene (69.98%), eugenol (12.98%), α-selinene (5.93%), and other compounds.

5. Discussion

The oil yields from each herb provide insights into the abundance of oil glands or droplets present within their respective structures. *Ocimum tenuiflorum* L. leaves exhibited a yield percentage of 0.13%, suggesting a relatively lower density of oil glands within glandular trichomes in the epidermis. In contrast, *Curcuma longa* L. (rhizome) displayed a slightly higher yield percentage of 0.1875%, indicating a moderate presence of oil droplets within the parenchyma cells of the rhizome tissues. Notably, *Citrus hystrix* DC leaves demonstrated a significantly higher yield percentage of 0.93%, suggesting a relatively larger number of oil glands consisting of secretory cells or idioblasts that accumulate essential oils within the plant's structure.

The GC-MS analysis of this study showed the active compounds of the three herbs. In *Curcuma longa* L., S Rafatullah (1990) studied the ethanol extract of turmeric in rats for its ability to inhibit gastric secretion. The turmerone group exhibits therapeutic properties that are beneficial for treating stomach ulcers. Promoting wound tissue growth and stimulating mucin secretion to coat the stomach accelerates the healing process (Rafatullah et al., 1990). Joerg Hucklenbroich et al. (2014) investigated the properties of ar-turmerone through in vitro and in vivo studies. Hucklenbroich's study found that ar-turmerone significantly increases the proliferation of neural stem cells (NSCs) in vitro, promoting neuronal differentiation of NSCs both in vitro and in vivo, and mobilized proliferating NSCs from specific brain regions (Hucklenbroich et al., 2014). Followed by *Citrus hystrix* DC., containing 6-octenal, 3,7-dimethyl-, (R)- and citronellol. Kazuhiko Nakahara et al. (2013) conducted a vapor-agar contact method to investigate the antifungal activity of volatile compounds, including 6-octenal, 3,7-dimethyl-, (R)- and citronellol, against various fungal strains. Nakahara's study found that the three compounds have antimicrobial properties against various bacteria and fungi strains (Nakahara et al., 2013). *Ocimum tenuiflorum* L. has caryophyllene and eugenol, which are key constituents with notable antioxidant activity, as demonstrated by the DPPH radical scavenging assay. Additionally, at a concentration of 10 µl/ml, basil essential oil exhibited remarkable free radical elimination by 90.84±1.79%. Furthermore, it possesses carminative properties and inhibits diarrheal bacteria, promoting bile expulsion (Veerapan, & Khunkitti, 2011). Solmaz Mohammadi Nejad et al. (2017) conducted a study on eugenol involved in vitro experiments assessing metabolites with aromatic hydroxyl groups. Eugenol has antimicrobial properties, damages bacterial membranes, and enhances antibiotic penetration (Nejad et al., 2017).

In the Indian variant of *Curcuma longa* L. studied by Fuloria and colleagues, key bioactive compounds such as ar-turmerone (40%), α-turmerone (10%), and curlone (23%) were identified. Contrary to this, while the Thai variant exhibited similar percentages for curlone and ar-tumerone, α-turmerone was notably absent. Furthermore, analysis of *Ocimum tenuiflorum* L. from India revealed significant disparities in the composition of eugenol (ranging from 50% to 57%), caryophyllene (22% to 32%), and methyl eugenol (6% to 14%) compared to Thai samples. The divergence continued with holy basil, where the eugenol content in Indian samples ranged from 50% to 57%, contrasting sharply with Thai variants at 12.98%. Similarly, carophyllene constituents in Thai holy basil were substantially higher (69.98%) than those in Indian varieties (22% to 32%), with the absence of α-selinene noted in Indian samples. *Citrus hystrix* DC. from India, as studied by Siti *et al*., exhibited citronelol, while its Thai counterpart showcased 6-Octenal, 3,7-dimethyl-, (R)-, and 3-carene alongside other unique constituents. Despite some similarities in bioactive compounds between Indian and Thai herbs, distinct compounds were found exclusively in each region. The chemical compositions of herbs in Thailand and India may differ due to variations in environmental factors such as climate, soil type, altitude, and rainfall patterns. These diverse conditions can influence plant growth, metabolism, and secondary metabolite production, leading to differences in the bioactive compounds present in the herbs.

Herbal essential oils and herbal medicine, in general, are fields not fully understood, and despite their widespread use, they could potentially cause harm to the human body. Divya Singh et al. (2018), a member of the Faculty of Pharmacy at Babu Banarasi Das National Institute of Technology and Management, and her colleague have conducted a systematic review covering the safety concerns over herbal medicine. The review showed that there were possible toxic effects from the consumption of herbal medicine, especially when individuals don't know the chemical composition. The authors advise creating awareness among the general public regarding the use of herbal medicines and their potential toxic effects (Singh et al., 2018). This aspect is significant due to the prevalent utilization of herbal medicine in rural areas, characterized by inadequate comprehensive research and understanding of herbal remedies. The likelihood of individuals in rural settings consuming herbal medicine based on perceived effects is high, potentially exposing them to harmful substances. The ingestion of these toxic components in herbal remedies poses health risks, potentially resulting in various healthcare issues and contributing to a rush of avoidable patients. Therefore, by understanding the constituents and their therapeutic effects, individuals can safely incorporate herbal remedies, including essential oils, into their health routines, promoting overall well-being.

6. Conclusion

This study identified the chemical active compounds within three commonly found Thai herbs: turmeric (*Curcuma longa* L.), holy basil (*Ocimum tenuiflorum* L.), and kaffir lime (*Citrus hystrix* DC.). These herbs, known for their aromatic and pharmacological properties derived from their essential oils, were collected from central Thailand and referenced through the BKF botanical database. We extracted the essential oils from each herb using the water distillation method, followed by Gas Chromatography-Mass Spectrometry Analysis (GC-MS) to identify the chemical constituents. The study's findings revealed varying yield percentages, with kaffir lime boasting the highest at 0.93% and holy basil the lowest at 0.13%. The differences in oil yield percentages among the herbs suggest variations in the prevalence of oil glands and oil droplets, providing insights into the botanical structure characteristic of each herb. Further analysis uncovered key constituents within the essential oils of each herb. Turmeric exhibited a significant presence in the turmerone group, known for its therapeutic properties in treating stomach ulcers. Kaffir lime's essential oil had 6-octenal, 3,7-dimethyl-, a compound recognized for its antifungal activities. Lastly, holy basil's essential oil contains caryophyllene and eugenol, compounds known for their notable antioxidant activities. The variance in chemical compositions between Indian and Thai herbs underscores the diverse bioactive compounds inherent to each region's botanical specimens. This diversity may arise from various factors, including differences in soil composition, climate, cultivation practices, and genetic variations among plant populations. Understanding these differences not only enriches our knowledge of herbal medicine but also emphasizes the importance of considering regional variations when studying medicinal plants. The findings contribute to our understanding of herbal medicine's role in traditional and modern healthcare practices. Further research into the chemical properties of these herbs is imperative, enabling a precise description of their effects. Such an understanding of their distinct attributes holds promise for enhancing public health outcomes. By harnessing the specific therapeutic traits of these herbs, nations may see advancements in population well-being.

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