COMPARISON OF THIN LAYER CHROMATOGRAPHY (TLC) PROFILES OF *Curcuma xanthorrhiza, Curcuma longa* AND *Curcuma caesia* (FAMILY ZINGIBERACEAE) USING METHANOL EXTRACT

by

AIN NURNADIAH ATHIRAH BT AZAHAR

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DECLARATION

I declare that this thesis entitled "Comparison of Thin Layer Chromatography (TLC) Profiles of *Curcuma xanthorrhiza*, *Curcuma longa*, *Curcuma caesia* (Family Zingiberaceae) Using Methanol Extract" is the result of my own research except as cited references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

Name : Ain Nurnadiah Athirah bt Azahar

Date : /09/2025

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Lastly, I dedicate this humble work to my brave and resilient Palestinians. Your strength and hope have deeply moved and inspired me. You are not forgotten.

Comparison of Thin Layer Chromatography (TLC) Profiles of *Curcuma xanthorrhiza*, *Curcuma longa*, *Curcuma caesia* (Family Zingiberaceae) Using Methanol Extract.

ABSTRACT

This study aims to compare the Thin Layer Chromatography (TLC) profiles of methanol extracts from three *Curcuma* species: *Curcuma xanthorrhiza*, *Curcuma longa*, and *Curcuma caesia*, belong to the Zingiberaceae family. These species are known for their medicinal properties and complex phytochemical compositions, yet systematic comparative profiling using TLC remains limited. The rhizomes of each species were cleaned, dried, ground into powder, and extracted using Soxhlet extraction with methanol as the solvent. The crude extracts were concentrated using a rotary evaporator and diluted to different concentrations for analysis. TLC plates were prepared using silica gel as the stationary phase and a mobile phase composed of chloroform and ethyl acetate (6:4 ratio). The results revealed variations in the number, colour, and retention factor (Rf) values of the spots across the three species. *C. longa* exhibited the most distinct spots with bright yellow-orange coloration, indicating a high curcuminoid content. *C. xanthorrhiza* showed yellowish-brown spots, while *C. caesia* displayed darker brown to greyish spots, possibly due to aromatic compounds such as turmerones. This study demonstrates that TLC is an effective preliminary screening method to differentiate bioactive compound profiles among *Curcuma* species, providing valuable insights for pharmaceutical and cosmetic applications.

Perbandingan Profil Kromatografi Lapis Nipis (TLC) Bagi *Curcuma xanthorrhiza*, *Curcuma longa* dan *Curcuma caesia* (Famili Zingiberaceae) Menggunakan Ekstrak Metanol

ABSTRAK

Kajian ini dijalankan bagi membandingkan profil Kromatografi Lapis Nipis (TLC) bagi ekstrak metanol daripada tiga spesies *Curcuma* iaitu *Curcuma xanthorrhiza*, *Curcuma longa*, dan *Curcuma caesia* yang tergolong dalam famili Zingiberaceae. Ketiga-tiga spesies ini dikenali dengan nilai perubatan yang tinggi serta kandungan fitokimia yang kompleks, namun kajian perbandingan secara sistematik menggunakan kaedah TLC masih kurang dijalankan. Rimpang bagi setiap spesies telah dibersihkan, dikeringkan, dan dikisar menjadi serbuk halus sebelum diekstrak menggunakan kaedah pengekstrakan Soxhlet dengan metanol sebagai pelarut. Ekstrak kasar yang diperoleh kemudiannya dipekatkan menggunakan penyejat berputar dan dicairkan kepada kepekatan yang berbeza untuk tujuan analisis. Plat TLC disediakan menggunakan gel silika sebagai fasa pegun dan sistem fasa bergerak yang terdiri daripada pelarut kloroform dan etil asetat dalam nisbah 6:4. Hasil analisis menunjukkan terdapat perbezaan yang jelas dari segi bilangan, warna, serta nilai faktor tahan (Rf) tompok-tompok yang terhasil dalam kalangan ketiga-tiga spesies. *Curcuma longa* menunjukkan tompok yang paling ketara dengan warna kuning jingga terang, menandakan kehadiran kurkuminoid pada kepekatan tinggi. *Curcuma xanthorrhiza* menghasilkan tompok berwarna coklat kekuningan, manakala *Curcuma caesia* menunjukkan tompok yang lebih gelap, iaitu dari coklat ke kelabu, berkemungkinan disebabkan oleh kehadiran sebatian aromatik seperti turmeron. Secara keseluruhannya, kajian ini membuktikan bahawa kaedah TLC berupaya menjadi alat saringan awal yang efektif dalam membezakan profil sebatian bioaktif dalam kalangan spesies *Curcuma*, serta berpotensi memberi sumbangan penting dalam bidang farmaseutikal dan kosmetik.

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LIST OF ABBREVIATIONS

TLC	Thin Layer Chromatography
C. xanthorrhiza	*Curcuma xanthorrhiza*
C. longa	*Curcuma longa*
C. caesia	*Curcuma caesia*
UMK	Universiti Malaysia Kelantan
UV	Ultraviolet
CRM	Curcumin Reference/Standard Marker
LC-MS	Liquid Chromatography–Mass Spectrometry
rpm	Revolutions Per Minute
°C	Degrees Celsius
Rf	Retention Factor

LIST OF SYMBOLS

L1 *Curcuma longa* first bottle sample

L2 *Curcuma longa* second bottle sample

L3 *Curcuma longa* third bottle sample

X1 *Curcuma xanthorrhiza* first bottle sample

X2 *Curcuma xanthorrhiza* second bottle sample

C1 *Curcuma caesia* first bottle sample

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Carl Linnaeus, a Swedish physician and naturalist who codified nomenclature (the contemporary practice of naming organisms) and created the genus *Curcuma* (Nair, 2013). The generic name which is *Curcuma* originates from the Arabic word "*kurkum or saffron*", that comes from the resemblance of the plant's rhizome with saffron (Ibrahim et al., 2021). Several species are indigenous to regions of the Indian Oceans, Northern Australia, India, Southeast Asia, and Southern China (Sasikumar, 2005). Those species are *Curcuma amada* (Mango Ginger), *Curcuma longa* (Common Turmeric), *Curcuma aromatica* (Wild Turmeric) and *Curcuma xanthorrhiza (*Javanese Turmeric) (Cristobal et al., 2021).

One of the plant species in the Zingiberaceae family, commonly referred to as the ginger family, is *Curcuma xanthorrhiza* (Larsen, 1998). Zingiberaceae is the largest family in the order of Zingiberales which mainly consist of eight families (Kress et al, 2001). Starting with four species of bananas, they are classified as the Lowiaceae (Syauqina, 2019), Heliconiaceae (Coleby-Williams, 2021), Musaceae (Coleby-Williams, 2021), Strelitziaceae that is known as Mandela Gold (Hodgson, 2020), and for the ginger family, those four families are Zingiberaceae (Simpson, 2010), Marantaceae (Lianah et al., 2010), Costaceae and Cannaceae (Coleby-Williams, 2021).

Family Zingiberaceae comprises about 54 genera and 1400 species of aromatic perennial herbs with a few traits of creeping horizontal and tuberous rhizomes (Alolga et al., 2022).

Those species include 200 species of *Alpinia*, 110 species of *Etlingera*, 50 species of *Hedychium* and 65 species of *Amomum.* which are also widely found and dispersed over the Americas, Asia, and Africa, as well as other tropical and subtropical regions (Boonma, 2023). *Curcuma xanthorrhiza* acquires several names according to its regions such as *Temulawak,* Java turmeric in Sunda, Indonesia and Malaysia while *Temu Lambak* in Madura, Indonesia (Dewi, 2022). Alkaloids, curcumin, and tannins are among the chemical components found in *C. xanthorrhiza* that are used in traditional medicine to treat digestive ailments, asthma, and diarrhea (Kustina, 2020).

One of the research projects has been conducted about how *C. xanthorrhiza* can be one of the components in increasing the antimycobacterial effects on the oral health through its formulation of the oil using different sonication times (Cho et al., 2023). This research will be discovering more about the active compounds in the different parts of *C. xanthorrhiza* through the thin layer chromatography (TLC) which will differentiate the whole function and structure of the plant to the regular turmeric (Ali Miftahudin et al., 2011). In TLC, the stationary phase is usually build using ground alumina or silica particles with a thin layer of the specific stationary phase, produced on a glass slide by coating the adsorbent with it (Avello, 2020).

The use of *C. xanthorrhiza* has been widespread in the cosmetics and skincare industry specifically in anti-aging, whitening and acne inflammation since decades ago (Ali Mahmoud et al., 2017). The rhizome of this plant is also one of the main ingredients for the *jamu* formulation that has been widely used in Indonesia as a dietary and medicinal herb (Rahmat, 2021).

Curcuma longa is known as Common Turmeric which has been very functional in medicinal use for about 400 years (Prasad & Aggarwal, 2011). *C. longa* is also act as the main component in pharmacological activity and medicine (De Oliveira Filho et al., 2021). *C. longa* contains more than 100 chemical compounds found that contribute to its healing abilities (Tian et al., 2025). *C. longa* has been used in traditional medicine to reduce hay fever, anti-inflammatory and indigestion. This plant species is believed in its capabilities of preventing cancer by slowing the development of new blood vessels in tumors and their metastases (BSc, 2023). The color of the main rhizome is yellowish brown with a dull orange in the inner part which is 2.5 cm with small tubers (Prasad & Aggarwal, 2011). The production of the *C. longa* as the medicine starts by drying down the rhizome of the *C. longa a*nd when the rhizome is completely being dried, but can be pulverized to a powder form with a bitter and sweet flavor (Fuloria et al., 2022).

Curcuma caesia Roxb is often referred as Black turmeric, is a perennial herb that is included in the family Zingiberaceae (Haida et al., 2022). This plant is extensively found in the Papi Hills of East Godavari and in northeastern and central India (Kamarul Zaman et al., 2013). The rhizome size is tuberous with the diameter 2-6 cm, and contains a camphoraceous sweet odor (Sahu et al., 2016). Both fresh and dried *C. caesia* rhizomes are used in traditional medicine to treat bronchitis, leucoderma, asthma, and tumors. Essential oils mainly consist of camphor, borneol, ar-turmerone chemotype, and 1,8-cineole are the main component in formulating the aromatic rhizomes and leaves (ProdyutMondal, 2013).

C. caesia also can be one of the main dishes and consumed as salad or *ulam* in Kota Belud, Sabah by the Sama-Bajau people and the leaves are freshly eaten or can be medicine to treat cough or consumed fresh (Ibrahim et al., 2023). Despite its use in human medicine, *C. caesia* is also employed in animal medicine. Cattle with gastrointestinal disorders are given the rhizome juice and mustard oil on an empty stomach for two to three days (Ibrahim et al., 2023).

Thin layer chromatography (TLC) will be the main method to identify the compounds of the three species of *Curcuma* which is *Curcuma xanthorrhiza*, *Curcuma longa and Curcuma caesia.* This method was first reported used by a Russian scientist, Mikhail Tsvet in 1903. TLC is a chromatography technique used to separate compounds of mixture using a thin stationary phase. Through this technique, preliminary screening can be carried out to identify the different types of compounds in the mixture (Firas, 2015).

1.2 Problem Statement

Curcuma xanthorrhiza, *Curcuma longa* and *Curcuma caesia* are the herbaceous plants that can help and contribute to health and wellness (Chen, 2012). These plants have been scientifically proven years ago that the bioactive compounds found will be useful and effective to treat many diseases such as kidney failure, stomach illness and arthritis (Rahmat, 2021). Nevertheless, there is no research conducted studying the comparisons between these three species of *Curcuma* have been established hence it is hard to distinguish the differences between their similar morphological characters.

Besides, most of the studies also only emphasize the importance of one of the species in treating any health illness hence there is a gap of the research about the difference between *C. xanthorrhiza*, *C. caesia and C. longa.* Moreover, each of the plants such as the rhizomes, leaves and the flowers contain different amounts and types of compounds that can contribute to any disease's treatment components (Chand, 2017).

1.3 Objective

The objective of this study is to compare the thin-layer chromatography (TLC) profiles of methanol extract for *Curcuma xanthorrhiza*, *Curcum*a *longa* and *Curcuma caesia.*

1.4 Scope of Study

This study was conducted at the Microbiology and Biochemistry Laboratory, Faculty of Earth Science which is located at Universiti Malaysia Kelantan (UMK), Kampus Jeli. The rhizomes of *Curcuma xanthorrhiza*, *Curcuma longa* and *Curcuma caesia* that were planted in Nursery ginger and herbs, institute of food security and sustainable agriculture (IFSSA) in UMK Jeli Campus, were collected and brought to Microbiology and biochemistry laboratory, Faculty of earth science, UMK Jeli Campus for the research purpose and the preparation of the samples was started by drying and powdering down the plant in the laboratory. The process of thin-layer chromatography (TLC) is important to identify and compare the selected profiles by using methanol extraction was executed in the laboratory of UMK, Kampus Jeli.

1.5 Significance of Study

The importance of the data that will be collected from this study is to assist in identifying the differences of TLC profiles of selected *Curcuma* species which are Curcuma *xanthorrhiza*, *Curcuma Longa* and *Curcuma caesia*. The composition of any bioactive compounds that will be found can be useful to differentiate the *C. xanthorrhiza*, *C. longa* and *Curcuma caesia* (Prasad, 2010). The outcome of this research will help in elevating the biological function of these three species in cosmetics and pharmaceuticals as the capabilities and the differences of the compounds exists have been scientifically proven (Oon et al., 2015). On the other hand, this study will be useful to any entrepreneurs and cosmetics manufacturer who is interested in exploring and starting the skincare business featuring the traditional and herbal plants in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical Description of *Curcuma xanthorrhiza*, *Curcuma longa* and *Curcuma caesia*

C. xanthorrhiza, *C. longa* and *C. caesia* are the species from order Zingiberales and belong to the Zingiberaceae family which is the largest family of Zingiberales (Figure 2.1) (Prince & Kress, 2002). The Zingiberaceae family was earlier classified into the subfamilies Costoideae and Zingiberoide which were subsequently given the independent family status as Costracea and Zingiberaceae (Nair, 2013). Zingiberales went a proposed rapid radiation in the Cretaceous, resulting in eight families of the order which is the Lowiaceae (Syauqina, 2019), Heliconiaceae, Cannaceae (Coleby-Williams, 2021), Musaceae, Strelitziaceae, Zingiberaceae (Simpson, 2010), Marantaceae, Costaceae (Lianah et al., 2010). Zingiberaceae comprises 52 genera and more than 1300 species of monocotyledon plants (Benedict, 2010). In addition, the Family Zingiberaceae has very diverse morphological characteristics in the seedling and embryo structures (Benedict et al., 2015). The Family Zingiberaceae has four subfamilies which are Siphonochiloideae, Tamijioidae, Alpinioideae and Zingiberoideae. These four tribes are divided by their number of fertile stamens and staminodes, number of ovules per locule, number of locule per anther and the embryo's shape (Lining, 2016).

Genus *Siphonochilus* is one of the genus under the subfamilies Siphonochiloideae while Genus *Tamijia* is the genus under the subfamily Tamijioidae (Schuman, 2019). From sub family Zingibereroideae, there are two genus that belong to its genus *Curcuma* (turmeric) and Genus *Zingiber* (ginger) (Buchheim, 1963). Genus *Curcuma* was clarified by Linneus in his Species Plantarum (Linnaeus, 1753). The name originates from the word "Kurkum," in Arabic and it indicates the yellow color and *Curcuma* is the Latinized version (Ravindran, 2007). *Curcuma* was first explained early on 1678-1693 by Van Rheede in his *Journal of Hortus Indicus Malabaricus* (Nair, 2013). Every plant in the genus *Curcuma* is usually aromatic, yet some species have a distinctively odd scent (Poudel et al., 2022).

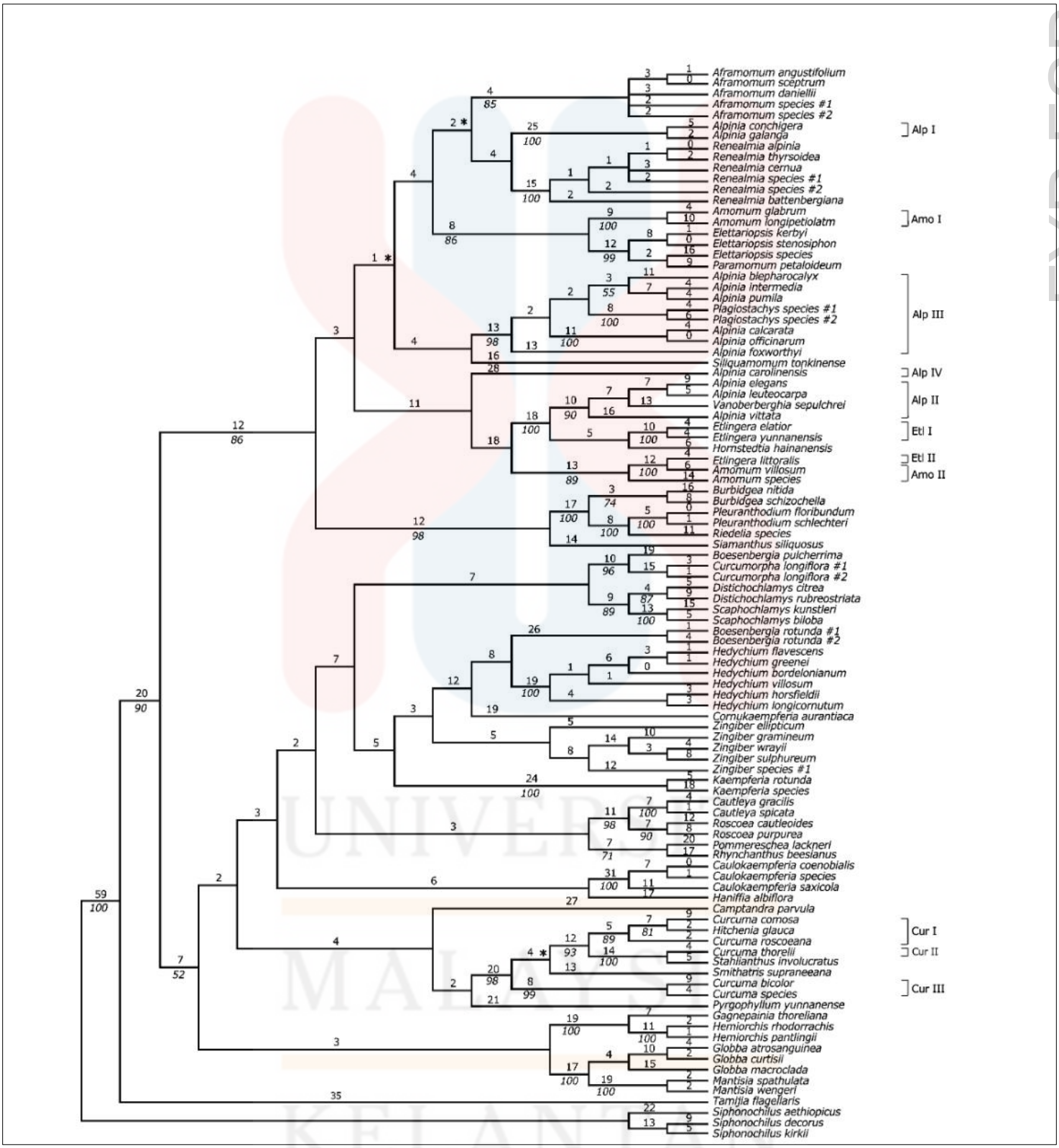

Figure 2.1 Phylogenetic tree of Family Zingiberaceae (Prince & Kress, 2002)

Conical or ellipsoid tubers are frequently found in the roots of *Curcuma* plant species that are connected to the rhizome (Kress et al., 2013). Typically, triploid in structure, these plant species reproduce asexually by rhizomes (Figure 2.2a). A lot of studies conducted have been proving the differences in morphological traits of the plant species during the growth stages. Basal leaves blades are normally broad in shape and rarely linear in shape and the flower contains single anther and large compound spikes which is a prominent trait in identifying the plant from the genus *Curcuma* such as *Curcuma longa* (Figure 2.2b) (Singh et al., 2024).

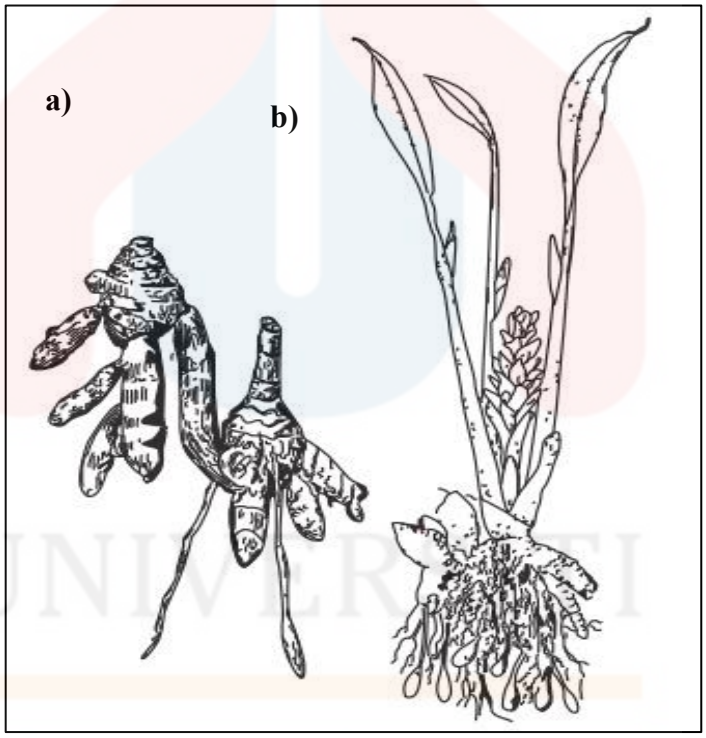

Figure 2.2a) Rhizomes of Curcuma longa, the main source of antioxidant which is curcumin **2.2b)** Morphology of Curcuma longa that is widely grown for its benefits in medicinal and culinary uses (Turmeric, 2022)

2.2 Distribution of *C. xanthorrhiza*, *C. longa* and *C. caesia*

2.2.1 *Curcuma xanthorrhiza* Roxb.

C. xanthorrhiza is a plant species from Indonesia and also mainly cultivated in Sri Lanka, the Philippines, Malaysia and Thailand (Azemi, 2023). This plant may thrive up to 2500 meters above sea level in the lowlands. According to the Rahman et al., (2021). *C. xanthorrhiza* can be found in almost all of Indonesia's major islands, including Java, Sumatra, Kalimantan, and Maluku.

C. xanthorrhiza is widely grown in many parts of India such as West Bengal, Gujarat and Karnataka as it is a native species in north India (Priyadarshini & Sahu, 2024). In addition, *C. xanthorrhiza* also can be found in Taiwan, Japan and China (Zhou et al., 2016). Based on Yurasbe et al., (2023), In Malaysia, the distribution of the *C. xanthorrhiza* is mostly found in Kedah, Johor and Penang as it is famous for its usage in herbal medicine. Furthermore, more than 19 genera and 200 species have been reported from Borneo. The map displays the global spread of the distribution of the *C. xanthorrhiza* around the world region with Indonesia (Figure 2.3).

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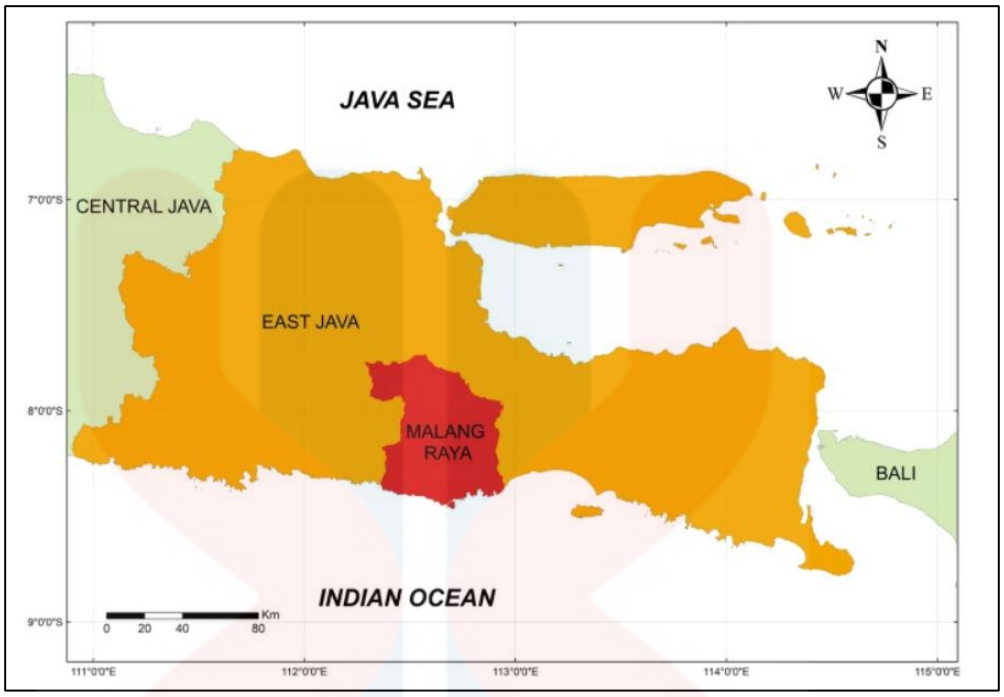

Figure 2.3 Location of traditional herbal drink processing observations in Malang Raya, East Java, Java Island, Indonesia (Estiasih et al., 2025).

2.2.2 *Curcuma longa* Roxb.

C. longa is commonly found in the region of Southeast Asia, southern China, New Guinea and Northern Australia. It is also naturally found in some warm regions such as tropical Africa, Central America and many Islands in the Pacific and Indian ocean (Iweala et al., 2023). Based on Yurasbe et al. (2023b), in Malaysia, *C. longa* is a plant that grows abundantly especially in tropical and subtropical locations that offer the warm, humid atmosphere needed for its growth. Small-scale farmers frequently grow it in rural and agricultural areas in Peninsular Malaysia, which includes states like Perak, Pahang, Kelantan, and Terengganu.

Malaysia's tropical climate is ideal for growing this plant because it grows best in well-drained, fertile soils with enough rainfall. In addition to being cultivated for traditional and commercial uses, *C. longa* is also frequently found in home gardens, particularly in rural areas where it is prized for its cultural and medicinal worth in addition to its culinary use.

Meanwhile, India is the world's greatest producer, consumer and exporter of *C. longa*, and it is widely grown and disseminated throughout the country. States like Andhra Pradesh, Tamil Nadu, Telangana, Karnataka, Maharashtra, Odisha, and West Bengal are the main locations for the crop's cultivation because of their warm, humid climates and fertile loamy soils, which are perfect for its growth (Dixit & Awasthi, 2009). *C. longa* extensive cultivation in rural and semi-urban areas has been maintained by its strong integration with Indian agriculture, culture, and traditional medicine, especially in Ayurvedic traditions (Dolase & Chaudhari, 2024)

2.2.3 *Curcuma caesia* Roxb.

C. caesia, known as *kala kadi,* is widely distributed in India, Nepal, Thailand, China and Bangladesh and Bhutan (Figure 2.4) (Orong et al., 2024). Based on Lenka et al. (2025), in Meghalaya, *C. caesia* is an important crop as it is cultivated by many farmers but they normally cultivated it without adding any nutrient source and sometimes they practically add some household waste. Therefore, the rhizomes produced are in very low quality and it lessens the production of this plant in the area.

In Malaysia, *C. caesia*, known as *kunyit hitam* is mostly found in Alor Setar, Kedah, Negeri Sembilan, Pahang and Kelantan. *C. caesia* is famous for its effectiveness as an anti-inflammatory (Haida et al., 2022). Research conducted by Ibrahim et al. (2023) explained about, its therapeutic properties and how this species usually grows in wet, dark areas like forest borders and undergrowth, however it is occasionally grown in cultivated herbal gardens.

Despite not being as extensively grown as *C. longa*, *C. caesia* is gaining popularity in Malaysia due to its traditional medicinal applications, especially for the treatment of skin disorders, inflammation, and discomfort. In Malaysia, local herbalists and traditional healers might grow it on a small scale, particularly in regions where ethnomedicine is still used. However, compared to other *Curcuma* species, its distribution is still restricted, in part because of its unique ecological requirements for growth and high demands.

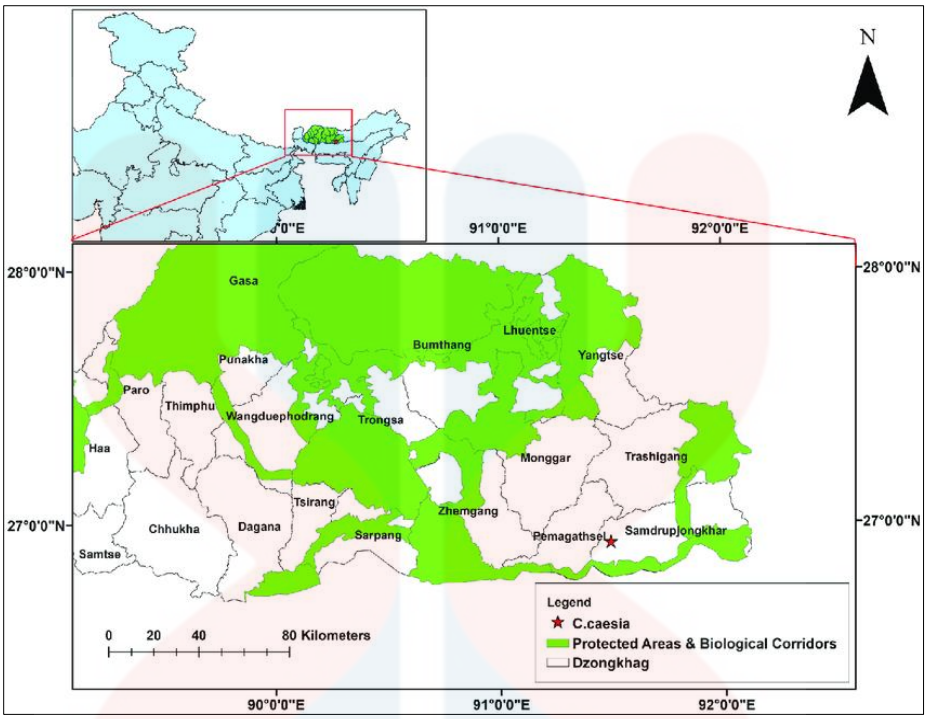

Figure 2.4 Distribution of *C. caesia* in Bhutan (Orong et al., 2024)

2.3 Morphology

2.3.1 *Curcuma xanthorrhiza*

Based on Oon et al. (2015b), *C. xanthorrhiza* has a rhizomatous herb 2m high and it is branched with the length 7-11 x 4-8 cm. The interior is deep bright orange to yellow orange, whereas the exterior is brownish orange. The leaves of *C. xanthorrhiza* are long and broad, measuring 50–55 cm in length and 18 cm in breadth and *C. xanthorrhiza* produces blooms that alternately emerge from the rhizomes (Figure 2.5a) (Patra et al., 2018). Flower stalks of this plant are 23 cm and consist of the leaves that correspond to the flower crown's length. (Figure 2.5b). The flowers that come out from the mother rhizomes are continuously blooming throughout the year.

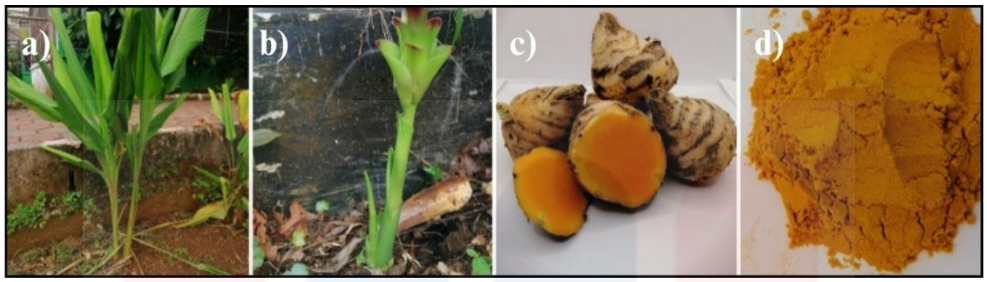

Figure 2.5 a) Mature leaves of *C. xanthorrhiza* growing in soil **b**) *C. xanthorrhiza* shoot emerging from the rhizome with flower bud development **c)** Harvested *C. xanthorrhiza* rhizomes, freshly cut to reveal the characteristic orange-yellow interior **d)** Ground powder, the dried and processed form commonly used as a spice and for medicinal purposes. (Rahmat et al., 2021).

Figure 2.6 Rhizomes of *C. xanthorrhiza* (*Curcuma xanthorrhiza*, n.d.)

Based on Windarsih et al. (2021), the color of the cut surface shows the rhizomes of *C. xanthorrhiza* are bright yellowish brown (Figure 2.5c). The outer color of the rhizome was almost similar among the *Curcuma* species. The rhizome, the primary component of *C. xanthorrhiza* is oval and circular like an egg, however the branch rhizome is elongated on the side. Besides, rhizomes of *C. xanthorrhiza* are usually dried and grounded to be used as medicinal herbs by the local communities (Figure 2.5d). (Rahmat et al., 2021). Each plant contains roughly three to four branch rhizomes, and its fibrous root systems has a length of 2.4 cm (Figure 2.6).

It has been recorded that the highest average diameter for mother rhizomes *of C. xanthorrhiza* is 5.5 cm which is the tallest among other *Curcuma* species (Figure 2.6). Based on the research conducted by Sutha Devaraj (2012), *C. xanthorrhiza* usually have a common flowering especially during the wet season, one of which commonly occurs in Penang, Malaysia between November and *C. xanthorrhiza* grows up to 750 m above the sea and it can be harvested after 8-12 months.

2.3.2 *Curcuma longa*

C. longa is an herbaceous perennial plant with branching, bright to orange color, cylindrical in shape and the fragrant rhizomes that can reach a height of one meter. The leaves are big and up to 1-2 cm long, with the dark green surface while the undersides are pale green which are placed in two rows and alternate (Tung et al., 2019). Based on Sharma et al (2024), The petiole of this plant ranges from 50 to 115 cm and the simple leaf is 76 to 100 cm. The flowers of *C. longa* are yellow-white, dense, short spike and measured 4-6 inches long (Figure 2.7).

Figure 2.7 Flower of *C. longa* that grows on a spike-like stalk that is 4-6 inches long and the petals are yellowish white in color (Chane-Ming et al., 2002).

Figure 2.8 Rhizomes of *C. longa*. The shapes of the rhizomes are cylindrical or oblong with the length 0.32 cm – 2.5 cm. The colors of the rhizomes are bright orange yellow to reddish and brownish yellow (Mans et al., 2019).

The rhizome, which is the horizontal stem that resembles a tuberous root, is fleshy with an ellipsoidal primary tuber at the base of each aerial stem that grows slightly curved when the plant is matured**.** The matured rhizomes are called fingers that are bright orange in color and give off a fresh and peppery smell (Figure 2.8) (Ibáñez & Blázquez, 2020). It also contains yellow sap that can leave a yellow stain on cloth or skin that might be impossible to remove (Kumar et al., 2014).

2.3.3 *Curcuma caesia*

Based on Thi et al. (2019), *C. caesia* grows with height from 0.5m -1.2 m and is divided into large rhizomes. The leaves of *C. caesia a*re usually present in the groups of 10-22 each and the leaves are broad and glabrous (Figure 2.9a). *C. caesia* flower usually bloom shorter than their bracts and have a pale yellow, reddish, and purplish outer border (Figure 2.9b). The flowers usually appear in June until July while the rhizomes usually mature in September and October (Baghel et al., 2013).

As the plant propagates with rhizomes, the tapering roots with the yellow and brown fibrous are present all over the surface of the rhizome (Figure 2.9c) (Kamaruzzaman et al., 2013). The rhizome is tuberous with a sweet scent and 2-6 cm long. The color of the rhizomes is dark brown, bluish black in color at the surface part (Figure 2.10). A number of variables including the growth environment and genetics, may contribute to the distinct characteristics of multiple accessions. (Sonjit Das et al., 2013).

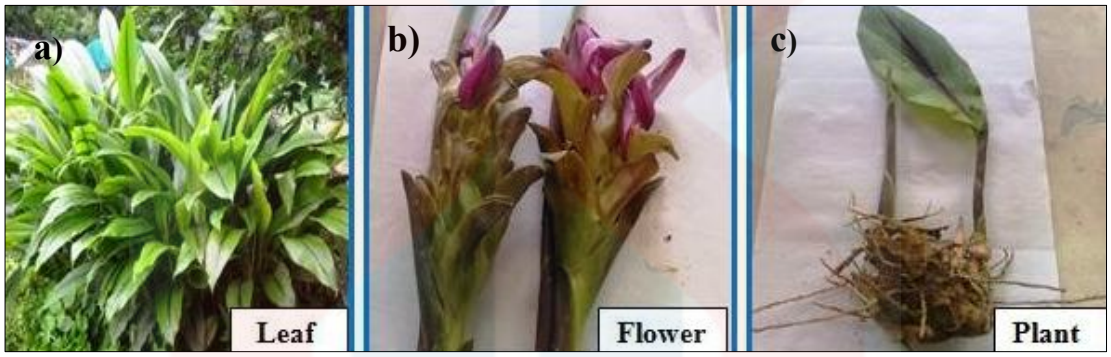

Figure 2.9 a) Leaf, flower and the plant structure of *C. caesia* **b)** The leaf of *C. caesia* that has a deep purple color in the middle part of the lamina **c)** Yellow and brown fibrous roots are present all over the surface of the rhizomes (Kamaruzzaman et al., 2013).

Figure 2.10 a) Dark brown, bluish black of the *C. caesia* rhizome**.** The shape of the rhizomes is ovoid and acute at the tip. The rhizome of this plant has a bitter, sharp and pleasant odor. It also has anti-bacterial and anti-fungal properties **(**Kamaruzzaman et al., 2013).

2.4 Medical Importance

2.4.1 *Curcuma xanthorrhiza*

C. xanthorrhiza have been reported and investigated about its antioxidant, anti-cancer, anti-inflammatory and anti-hepatotoxic. *C. xanthorrhiza* is also one of the best components in reducing cholesterol and increases the milk production during breastfeeding (Aimi, 2017). The natural anti-oxidant compounds such as curcumin and xanthorrhizol help in exerting on the anti-inflammatory effect by scavenging the oxygen species. Such as hydroxyl radical and singlet oxygen by chemically reducing the oxidized compound.

Besides, the cytotoxic actions of xanthorrhizol can contribute to the resistance against the growth of the cancer tumor (Simamora et al., 2022). Research conducted by Nurcholis et al. (2017), proved that the most well-known essential phytochemicals obtained from *C. xanthorrhiza* rhizomes are terpenoids and curcuminoids. Hence, the medicinal activity of this plant is mostly caused by these two groups of compounds.

C. xanthorrhiza has been widely used in traditional medicine (Oon et al., 2015). According to tradition, this plant's rhizome is typically eaten raw, steeped, or even as food (Rahmat, 2021). It also can be combined with other medicine such as the mixture of *C. xanthorrhiza* and *C. longa* is believed by the Javanese capable of increasing the immunity strength and maintaining a good excretory system. Moreover, the combination of a few species of herbal plants such as *Andrographis paniculata* (green chiretta) and *C. xanthorrhiza* can be the best cure for gastric disease and widely used by the people of Sulawesi (Van et al., 2025).

In addition to its anti-inflammatory properties, *C. xanthorrhiza* has shown antibacterial activity against a variety of bacteria and fungi, such as *Candida albicans* and *Staphylococcus aureus* (Nurcholis et al., 2024). It is frequently used to treat respiratory disorders, gastrointestinal disorders, and skin infections. Furthermore, by causing apoptosis and preventing the growth of cancer cells in models of liver and breast cancer, xanthorrhizol has demonstrated encouraging anticancer potential (Al-Amin et al., 2024). These results demonstrate *C. xanthorrhiza* is important for medical significance in both conventional medicine and contemporary pharmaceutical research.

2.4.2 *Curcuma longa*

There has been so much interest in the wide use of *C. longa* for certain health conditions such as rheumatoid arthritis and Alzheimer's disease (Hidayat et al., 2024). In traditional Chinese and Indian medicine, *C. longa* has long been used to aid digestion and to treat many diseases particularly as anti-inflammatory and for the treatement of jaundice, wound and hemorrhage (Labban, 2024). In India and Bangladesh, this plant species helps in asthma and cough when a study conducted by Boskabady et al. (2021), proved that *C. longa* therapy is able to lower the inflammation in the airways by reestablishing the balance between oxidants and antioxidants. The findings from this research also discovered that this plant had protective effects on lung diseases, oxidative stress and immunological illness.

Besides, *C. longa* also helps in improving asthma control in children and also adolescents (Becky & Becky, 2021). Curcumin, which is an antioxidant, helps in reducing bronchial-hyper-responsiveness and decreases inflammatory cytokine levels. This treatment was carried out by allowing a total of 34 children and adolescents aged 10-18 years old that suffered with persistent asthma to be enrolled in a clinical trial. They are assigned to ingest the powdered rhizomes of *C. longa* approximately around 500 mg/day for 7 to10 year olds and 750 mg/day for 11-14 years old and 1000 mg/day for 15-18 years. This controlled trial did bring a good result when most of the patients reported less nighttime awakening within three months. Overall, the evidence study showed that the powdered root of *C. longa o*ver a three months period can significantly improve asthma control among the children and adolescents.

C. longa is also discovered as one of the best medicinal plants used during postnatal care in traditional medicine by the Malay people. For example, Peninsular Malaysia such as Muar in Johor and Kuala Pilah in Negeri Sembilan (Jamal et al., 2011). The rhizomes of this plant species have been useful to treat wounds and being the main ingredient in *jamu,* medicated talcum powder and bathing solution. Based on the study conducted by Zumaidar et al. (2019), three to seven treatments, including body massage, herbal medicines, body scrubs, and bathing herbs, are often required for 40–46 days following childbirth for postpartum moms. These traditional treatment practices are solely to improve blood circulation and muscle recovery. The rhizome of *C. longa* that contains Curcumin compound can fasten the healing of uterine wounds and as an organ just by drinking the medicinal herbs (Hamlbar et al., 2025).

The second has postnatal treatments focusing on recovery treatments for vital organs which are in liquid and pill forms. Based on Haneef (2015), the rhizome will be grounded to the powder form (Figure 2.11) and then cooked with honey to be drunk while in the pill form (Figure 2.12), the pill will be given five pills in a day and five pills at night during the first 10 days of postnatal periods and these treatments were also documented among the Kerala communities in India.

Figure 2.11 Powder form of *C. longa* as of the main ingredient in traditional herbal remedies by Malay, India and Chinese around the world (Azeez & Lunghar, 2021).

Figure 2.12 Sample of *C. longa* in the tablet form consumed by the locals in Kerala, India (Admin, 2024)

2.4.3 *Curcuma caesia*

C. caesia is significant and well-known as traditional healer for many ailments as the rhizomes is used to cure dysentery and as poultice in rheumatic pain, sprains and bruises (Publisher, 2016). Besides, the dried rhizomes are known for its strong antioxidant and antifungal components that help in the digestive system. It will smoothen the digestion and stimulate the good functioning of the kidneys and livers (Pooja, 2021). Few studies recorded that oleoresins in *C. caesia* rhizome essential oil have high efficiency in resisting *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* from attacking the blood cells (S. Fuloria, 2022). Besides, the inhibition zone was observed and measured and it is effective in healing the infection of the wounds against *B. subtilis* (Paw et al., 2019). Based on Sharma et al., (2021), this plant species is also documented to be such a huge help in neuroscience research. This is proven when the rhizome of *C. caesia* consist of analgesic, the potential of antidepressants for the central nervous system is increased by their effects on muscle relaxants and locomotor depression.

The medicinal properties of *C. caesia* contribute to the high economic value and lead to the high demands, due to this biopiracy, this species is declared as one of the species that is threatened by the National Forest Departments of India (Chauhan, 2023). Research has been conducted by Neha Bahar (2013) focusing on the Comparative Phytochemical Screening of Bioactive Compounds in *C. caesia* and *C. longa* where *C. longa* has been proven that the presence of the phytoconstituents exist can treat several diseases.

Based on the research conducted by Paliwal et al. (2011), in his articles about Pharmacogenetic parameters for evaluation of the rhizomes of *Curcuma caesia*, the presence of alkaloids, steroids and tannins in *C. caesia* is the key component in curing any health infection illness. The rhizomes of this plant are packed with beneficial compounds like essential oils, flavonoids, alkaloids, and terpenoids, which give it a range of healing properties.

Traditionally, people have used it to treat inflammation, pain, wounds, and various skin problems. It also has antimicrobial and antifungal abilities, making it useful for fighting off infections. The essential oils, especially those containing ar-turmerone and camphor, are known to help with respiratory issues like asthma and bronchitis. Furthermore, steroids and tannins play such an important role in increasing the productivity of bioprotective energy in *C. caesia*. *C. caesia* is further known to support digestive health and is traditionally used to treat dyspepsia and flatulence. Its antioxidant properties help the body resist stress and maintain physiological balance.

2.5 Importance of Thin Layer Chromatography (TLC) in Drug Discovery

The quality control of crude extract and their bio compounds is important to confirm its identity and determination of its quality and purity (Edo et al., 2024). Hence, TLC is a separation technique that contributes in the chemical analysis in which the separation occurs in a layer of absorbent placed on an aluminum plate or plastic sheet and it is called a stationary phase (Santiago & Strobel, 2013). The separation occurs due to the difference in movement of the individual solutes under the influence of a moving solvent.

For example, there is research conducted to separate the combination of active ingredients contained in the non-prescription analgesic tablets (Pyka, 2014). The TLC medium is applied in small amounts to the bottom of the strip coated one side with a thin layer of silica gel along with the silicone that contains the primer. The actual separation in TLC happens when the molecules of a particular component in a mixture have high attraction for the adsorbent. This will lead to the slow movement of the compound while another component with less attraction will move rapidly during the mobile phase. The result of this analysis shows that aspirin is the most strongly attracted to the stationary phase while phenacetin is weakly attracted.

TLC is also important in the pharmaceutical field which is the manufacturing and engaging with drugs for medicinal purposes such as treating diseases also in diagnosis (Attimarad et al., 2011). Through this study, it also stated that TLC provides more information about the chromatographic information on a complex mixture of pharmaceutical and natural products hence it is widely used during the development stages of drug discovery.

This will definitely lead to maximizing the reliability of exciting techniques to cope up with the demands for better chemical analysis (Ciura et al., 2017). Apart from that, detecting and visualizing agents is also one of the crucial components to detect the type of the compound that exists in the substance to enhance the benefits and facilitate the research in bio compound identifications (Figure 2.13).

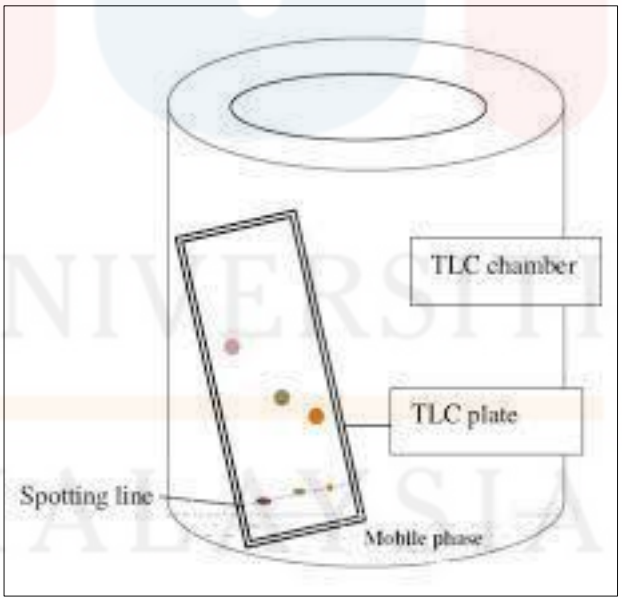

Figure 2.13 Mechanism component of TLC that consists of the TLC chamber, plate, spotting line and the mobile phase that will be the indicator of the movement of the different compounds (Aryal, 2023).

2.6 Soxhlet Extraction

Soxhlet extraction is one of the key methods in extraction as it will enrich the target compounds and remove any impurities, discovered in 1879 (Raynie, 2022). Heat energy is the main source of energy for this extraction procedure. It will require the heating process of the solvent to the reflux and siphon principle to extract the solid matter from the pure solvent (Zhang et al., 2023). Soxhlet extraction was first used during the extraction of fats and oil and as well as other nonpolar solutes and semisolid matches (Li et al., 2014).

Based on Q. Zhang et al (2018), Soxhlet extraction consists of few advantages in practicing it during the extraction method compared to the general soaking method. Research has been conducted focusing on observing the process of extracting the target compounds from fruits such as berries using a Soxhlet extractor (Borodulin et al., 2020). This research was carried out to study the ability of this extractor in reducing the cost of production while coping up with the growth of consumer and high demands of productivity. Water-alcohol, ethyl alcohol used as an extractant and the quality of the grain ethyl alcohol obtained from the extraction are equal to wheat and barley which is the highest quality of grains. The main advantage of this apparatus is that pure alcohol is fed inside the extractor with no saturated substance.

This will lead to a high volume of effective enrichment with aromatic oil and esters. This concludes that Soxhlet extraction can be one of the useful tools to obtain extract from various raw materials when the preparation time of tinctures was reduced from several weeks to several hours (Figure 2.14).

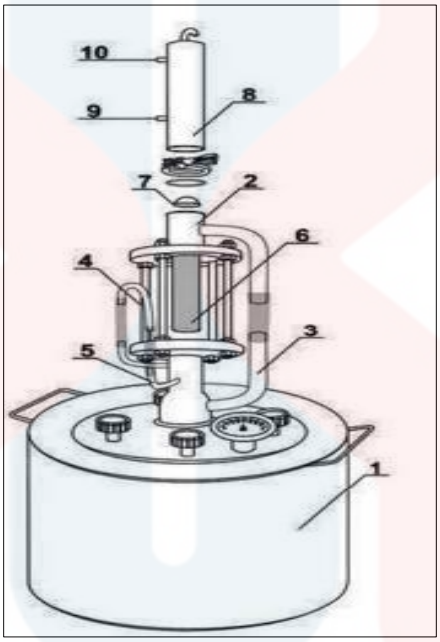

Figure 2.14 Soxhlet Extractor apparatus used during the extraction process (Borodulin et al., 2020). The first part with the label 1 is the distillation still, 2 is the extractor chamber, 3 is the distillation arm, 4 is the siphon with the arm that is labelled as 5. The sample will be placed in the thimble with the label 6,7 thimble cover, 8- reflux condenser, 9 is cooler nozzle and 10 is the cooler outlet nozzle.

Soxhlet extractor also plays an important role in plant studies where the extraction needs to be done with a very minimal amount of the respective plant samples. Based on the study conducted by Gopalasatheeskumar (2018), in his article entitled Significant Role of Soxhlet Extraction Process in Phytochemical Research, he stated that the Soxhlet procedure is also highly reproducible, which is a very crucial requirement in scientific experimentation where standardization and comparison of results between runs are required.

The extracts resulting from this process can be analyzed further using chromatographic and spectroscopic techniques for identification and determination of the active constituents. Its applicability to polar and non-polar solvents makes it have a wide application to a wide range of plant materials and phytochemical classes. Despite its effectiveness, the Soxhlet extraction method has several drawbacks. One major concern is the exposure to hazardous and flammable organic solvents, which can release toxic emissions during the process.

Additionally, Soxhlet extraction is not considered environmentally friendly, as it involves continuous heating, potentially contributing to air pollution (Naude et al., 1998). To address this issue, the use of a fume hood is recommended to prevent exposure or environmental contamination. Furthermore, the samples used for extraction must be dried and finely ground, and several parameters such as temperature, solvent ratio, and agitation speed need to be carefully controlled (M. Zhang et al., 2023b). Despite it being a conventional technique, the Soxhlet extractor is still a favorable method since it is affordable and simple to use, particularly for laboratories with limited access to advanced extraction equipment.

CHAPTER 3

METHODOLOGY

3.1 Collection of Rhizomes of *C. xanthorrhiza*, *C. longa* and *C. caesia*

C. xanthorrhiza, *C. longa* and *C. caesia* were harvested and collected from the nursery ginger and herbs, institute of food security and sustainable agriculture (IFSSA), Universiti Malaysia Kelantan (UMK) Jeli Campus (Figure 3.1). However, the preparation of the sample was conducted at Microbiology and biochemistry laboratory of UMK, Jeli Campus. The environment of these plant species was observed and recorded by capturing the plants with the ecological traits. The fresh sample preparation began by sorting out the rhizomes of the three species and removing any impurities such as soil (Kartini et al., 2021). Each of the plant rhizomes was cut into smaller pieces using a knife and cleaned under running water until all the dirt was removed (Figure 3.2). All the sectioned parts of the rhizomes were stored in clean and labeled zip lock bags according to their species before being brought to the microbiology and biochemistry laboratory, Universiti Malaysia Kelantan (UMK), Jeli Campus.

Figure 3.1 Nursery ginger and herbs, institute of food security and sustainable agriculture (IFSSA), UMK Jeli Campus.

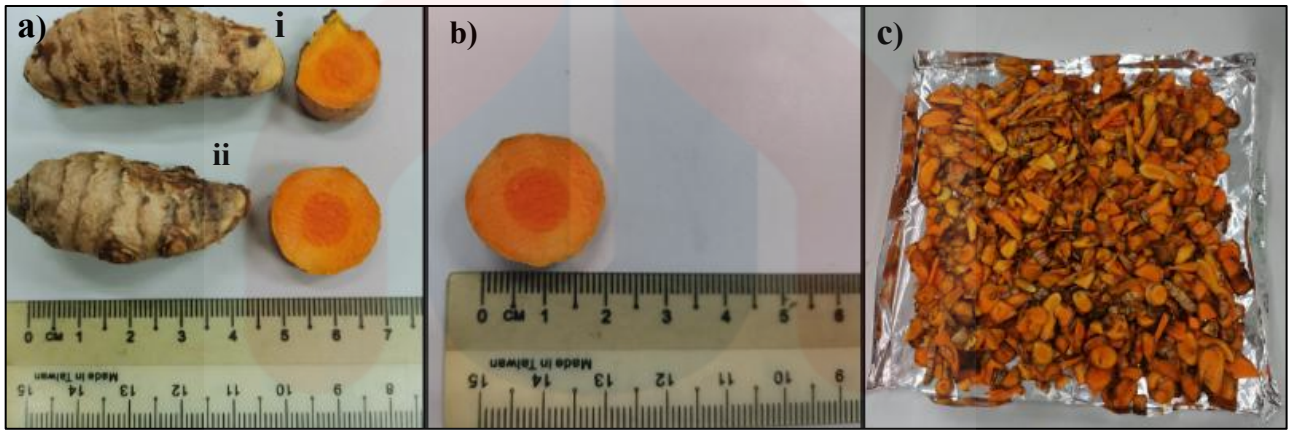

Figure 3.2 a) i) The rhizomes of *C. xanthorrhiza* and **ii)** *C. longa* of six and four cm **b)** One of the small pieces of *C. longa* that is range one to two cm **c)** Smaller pieces of *C. longa* that has been cut before drying with oven.

3.2 Preparation of *Curcuma xanthorrhiza*, *Curcuma longa* and *Curcuma caesia* Sample Powder and Methanol Extraction

The rhizomes of *C. xanthorrhiza*, *C. longa* and *C. caesia* were dried using a laboratory oven (BINDER, United States of America) that is provided by UMK, Jeli Campus. The temperature was set at 37 degrees Celsius (Alsaud & Farid, 2020). After the rhizomes were dried, a blender (Philips 600W, Netherlands) was used to grind the rhizomes into powder form in a shorter time (Figure 3.3).

This is very important because the powdered form of *Curcuma* has a greater surface area for better segregation of bioactive compounds from the plant powder (Pond, 2016). The sample then was weighted constantly using a weight scale (OHAUS Corporation, United States of America) to achieve the uniform weight for the three species of *Curcuma*. The zip lock bags containing the powdered rhizomes must be kept in the space with the room temperature which is 22 degrees Celsius (Aileen, 2016). The next step was to prepare the methanol extraction using the Soxhlet extraction method.

The powdered rhizomes of *Curcuma* were placed inside the thimble of Soxhlet extractor (BOECO, Germany). The thimble then was loaded into the main chamber of the Soxhlet extractor and the 200 ml of methanol (BIOCHEM CHEMOPHARMA, France) that is suitable for Liquid Chromatography Mass Spectrometry (LC-MS) as the extraction solvent. The methanol was filled into the 500 ml round bottom of the distillation flask before it was placed on the heating mantle (GONGYI YUHUA INSTRUMENT, China).

The Soxhlet extractor was clamped onto the top of the flask, and the reflux condenser was then placed at the top of the extractor. The round-bottom flask containing methanol was boiled at a temperature of 65°C (Hudaya, 2023). The heating and extraction processes were initiated by turning on the tap. After 25 minutes, the Soxhlet chamber became full, and the solution began to fill the round-bottom flask. The reflux process was repeated three to four times over a duration of two hours until the concentration of the solution in the flask gradually increased (Subramanian et al., 2011). In this study, the procedure was repeatedly conducted using the powdered form of three *Curcuma* species, and the entire extraction process was completed in approximately eight- ten hours.

A constant volume of 250 mL methanol was added weekly throughout the extraction process to prevent the extract from becoming overly concentrated and to ensure the reflux process was maintained efficiently. This step also prevented the methanol from evaporating completely during the Soxhlet extraction. Considering the safety during the extraction, it was also important that the heating mantle was set up in a well-ventilated area, preferably in a fume hood, to prevent the accumulation of flammable vapors (Figure 3.4). Proper ventilation was required, as methanol vapors were not only flammable but also unsafe to breathe in. An electric heating mantle provided a controlled and consistent environment and minimized the risk of overheating and fire or toxic exposure hazards (Rohman, 2020).

To obtain crude extract from the extraction process, the solvent extraction needed to be evaporated and dried through the rotary evaporator machine (YAMATO, Japan) (Figure 3.5) to remove the methanol and water. (Shanmuganathan, et al., 2022). The solution of the *Curcuma* for each species was needed to be half-filled in the 100 ml of round bottom flask and the receiving flask must be empty to avoid contamination (Donde, 2023). The water bath was being set up to 60 degrees Celsius and the boiling flask was connected horizontally to the evaporator of the rotary evaporator machine; the water bath temperature was set to 60 degrees Celsius to allow the methanol evaporation to occur efficiently and the condenser was monitored to ensure that the water vapor was flowing through it to indicate that the methanol is being removed from the samples.

The flask was rotated at 77 rotations per minute (rpm), a moderate speed that promoted even distribution of the extract along the inner wall of the flask, thereby increasing the surface area for evaporation. The water then was being removed from the samples by setting up the rotary evaporator machine temperature to 33 degrees Celsius. The water bath needed to be replaced to adjust the temperature to the lower temperature prior to the high temperature of methanol. These steps were repeated for *C. longa*, *C. xanthorrihiza* and *C. caesia*, the crude obtained was sticked out using spatula into the labelled universal bottles. The universal bottle contained the extract then was labelled and weighed before being put in the laboratory oven (BINDER, United States of America).

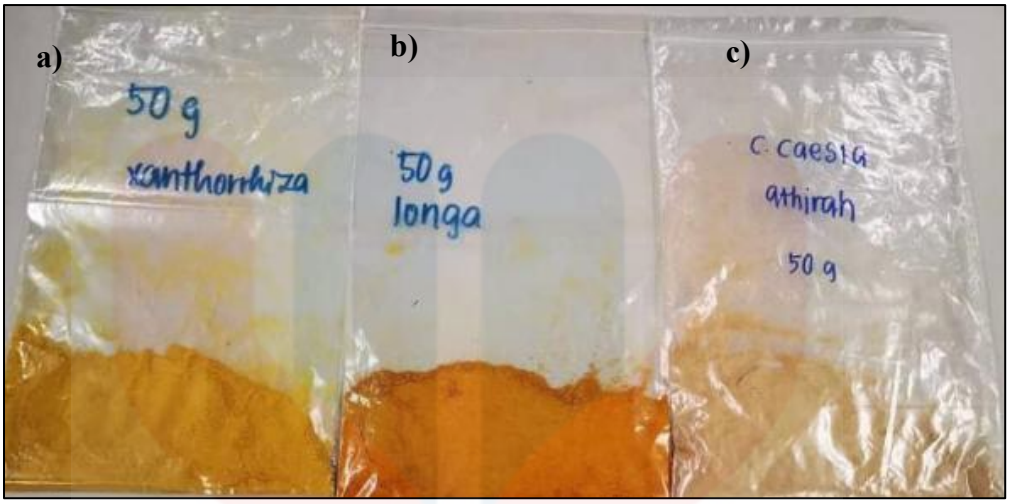

Figure 3.3 a) The powdered rhizome of *C. xanthorrhiza* showing a yellow-orange color **b)** The rhizome of *C. longa* with an orangey-red hue **c)** The powdered rhizome of *C. caesia* displaying a greyish-brown color.

Figure 3.4 Soxhlet Extraction set up in a fume hood showing the extraction of plant rhizome samples.

Figure 3.5 Rotary evaporator machine that was used in obtaining the crude extract of the *Curcuma* species (Crig, 2023).

3.3 Dilution of the *Curcuma* spp. Extract for The Sample Preparation

The 300 mg of each extract of the *Curcuma* obtained from the evaporation of the rotary evaporator machine was dissolved in 15 ml of methanol for the preparation of the stock solution. The process of dilution was repeatedly done for the three *Curcuma* samples until it achieves the desired concentration of the solution starting by creating the concentrations of 0 mg/mL, 5 mg/mL, 10 mg/mL, 15 mg/mL and 20 mg/mL using the micropipette and measuring cylinder. The preparation of the stock solution, using the certain volume of the methanol, was calculated using the formula as shown below (Table 3.3). Based on the Table 3.1, control solution containing only methanol was prepared during this experiment to serve as baseline measurement. This was important for the comparison of the diluted *Curcuma* stock solution. The cylindrical tube containing the dilution then was labelled according to the species (Figure 3.4) The formula of calculating the dilution was determined as below: $C_1V_1 = C_2V_2$, where

C_1 = concentration of the stock solution

V_1 = volume of the stock solution

C_2 = target concentration (e.g., 20, 15, 10, or 5 mg/mL),

V_2 =final volume of the diluted solution (5 ml).

Table 3.1 Table of dilution preparation for various concentration of *Curcuma* spp.

Target Concentration (mg/mL)	Volume of 20 mg/ml of the stock solution	Volume of Methanol (ml)	Final volume of the sample extraction (ml)
20	5.00	5.00	5.00
15	3.75	1.25	5.00
10	2.50	2.50	5.00
5	1.25	3.75	5.00
0	0.00	5.00	5.00

The dilution was being done by adding 1.25 ml of the 20 mg/mL stock solution and to complete the dilution, 1.25 ml of the stock solution was transferred into a clean container and 3.75 ml of methanol was added to reach a final volume of 5 ml. The solution was stirred using a well plate magnetic stirrer at 320 rpm and heated at 35°C to mix the sample evenly (THERMO SCIENTIFIC, United States of America) (Figure 3.6). The process of dilution was repeatedly done for the three *Curcuma* samples until it achieves the desired concentration of the solution starting by creating the concentrations of 0 mg/mL, 5 mg/ mL, 10 mg/mL, 15 mg/mL and 20 mg/mL using the micropipette and measuring cylinder.

Figure 3.6 The diluted extract of *C. caesia* was being stirred, resulting in a concentrated brownish-black colour.

3.4 Profiling Compounds of *Curcuma* spp. By Using Thin Layer Chromatography (TLC

During the Thin-Layer Chromatography (TLC), the silica gel was used to coat the plate for the stationary phase (Amara et al., 2024). The straight line was drawn 2.0 cm from the bottom of the plate gently using pencil on the plate. In this study, six dots were evenly drawn on the line representing the three species of the plant samples, the standard solution of CRM and the five different concentrations of samples. A line was drawn 3.0 cm from on the top of the silica plate and served as a solvent front. This line was functioned to determine the highest point the solvent reached.

Then, the sample solution of *C. xanthorrhiza* will be spotted on the second dot using the capillary tube 20 times after the standard solution of CRM, gently without scratching the silica gel 60 TLC plates (MACHEREY-NAGEL, Germany). These steps were repeatedly done for the other two samples which are *C. longa* and *C. caesia* using different capillary tubes to avoid contamination. To prepare the mobile phase, a total of 400 ml of solvent mixture was needed. This mixture was made by combining chloroform and ethyl acetate in a 6:4 ratio. Based on this ratio, 240 ml of chloroform and 160 ml of ethyl acetate were measured out using measuring cylinders. Both solvents were then poured into a clean beaker and mixed well to ensure they were fully combined. Once ready, the mixture was poured into a TLC development chamber until the solvent level was just enough to allow proper development of the TLC plate without touching the spots on the baseline.

After adding the mobile phase, the chamber was covered and left undisturbed for about five minutes. This allowed the inside of the chamber to become saturated with solvent vapors, which helps the solvent front move smoothly up the TLC plate (Figure 3.7). Once the chamber was saturated, a silica gel TLC plate was carefully placed inside using forceps, making sure the sample spots stayed above the solvent level. The lid was immediately closed again to prevent evaporation and maintain saturation.The TLC plate was left in the chamber for around 15 minutes to allow the solvent to rise up the plate by capillary action.

When the solvent front had reached an appropriate height, the plate was removed and left to air-dry. After drying, the developed plate was observed under a UV lamp set to 254 nm and through blue light using UV Transilluminator (MAJOR SCIENCE, United States of America (Figure 3.8). The spots that appeared on the plate were marked, and the Rf values were calculated. Each of the extracts was examined twice as a replication using the same technique to ensure that the thin layer chromatography (TLC) results were accurate.

Figure 3.7 The TLC plate was placed vertically in the chamber allowing the mobile phase to move via capillary action.

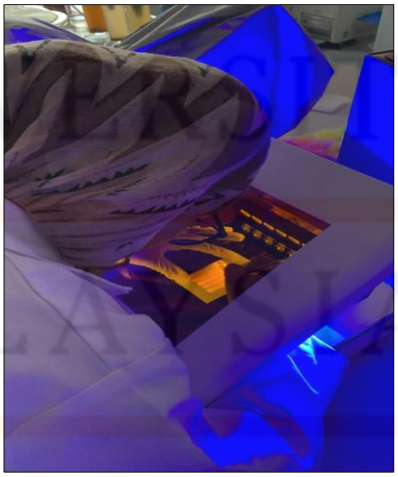

Figure 3.8 The spots were marked through the UV Transilluminator at λ 254 and blue light using pencil.

3.5 Analysis of Retention Factors Value (Rf) of *Curcuma* spp. Extract

The behavior of each compound was characterized by a quantity known as the retention factor (Rf)**.** The calculation began by measuring the distance the solvent had travelled from the baseline to the solvent front, as well as the distance the sample solution had travelled from the baseline to its respective spot (3.9) (Figure 3.9) (Kartini, 2021). The Rf value was then calculated by dividing the distance travelled by the compound by the distance travelled by the solvent on the silica plate (Grzelak et al., 2016).

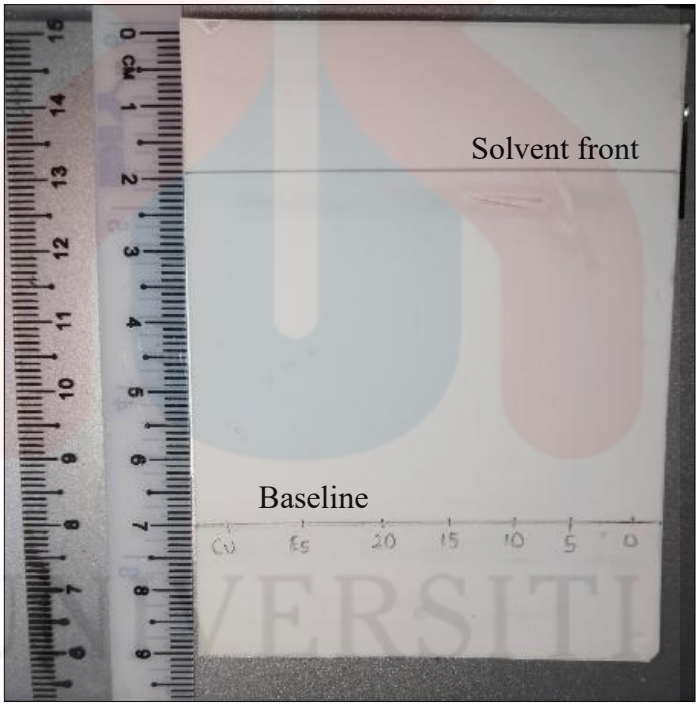

Figure 3.9 Solvent front is described as the point where the compound of the sample solution stops moving and the Baseline is the starting point where the sample is spotted (Kartini, 2021).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Weight Crude and Extract of *Curcuma* spp.

In the process of sample preparation, size reduction was performed to facilitate the extraction and the thin layer chromatography (TLC) analysis for three *Curcuma* species. After being cleaned and washed to remove any dirt, the rhizomes were weighted on the scale balance and the weights were recorded (Figure 4.1). Afterwards, the rhizomes were ground into fine powder to maximize the surface area for the extraction (Figure 4.2) (Nisrin, 2022). The table 4.1 below summarizes the weight of the rhizomes before and after grinding as the grinding process led to varying degrees of weight reduction, with *C. caesia* showing the highest weight loss percentage of 45%, followed by *C. xanthorrhiza* at 39%, and *C. longa* at 34%. The differences in the percentage loss could be attributed to the varying densities and water contents of the rhizomes, as well as the fibrous nature of the species (Pond, 2016).

C. caesia, known for its harder and more compact rhizomes, may have required more effort to break down during the grinding process, resulting in a higher weight reduction. On the other hand, *C. longa* exhibited a lower weight loss, suggesting it may have a more porous or softer structure, which ground more easily (Patra et al., 2023).

The weights of the crude extract were also recorded after the extract was dried down in the oven after removing the methanol through the rotary evaporator machine. The number of days to dry up were different according to the volume of the extract. For example, *C. longa* samples were divided into three universal bottles which were L1, L2 and L3 due to the higher amount of crude extract than other two species. The extract was recorded to achieve the constant weight after being dried down for 18 days. *C. xanthorrhiza* was labeled as X1 and X2 while *C. caesia* as C1. 12 days were taken for these two species to achieve the constant weights which both specifically were 5.73 g and 2.639 g (Table 4.2).

Table 4.1 Weight powder of three *Curcuma* spp.

Sample Species	Initial Weight (kg)	Final Weight After Grinding (kg)	Weight Loss (kg)	Percentage Loss (%)
Curcuma xanthorrhiza	1.0	0.61	0.39	39.17%
Curcuma longa	1.0	0.65	0.35	35.00%
Curcuma caesia	1.0	0.55	0.45	45.00%

Table 4.2 Crude extract of three *Curcuma* spp. weight.

Week	Weight (g)		
	C. longa	*C. xanthorrhiza*	*C. caesia*
1	37.904	22.928	10.556
3	28.000	17.000	7.800
6	18.500	11.500	5.400
9	13.000	7.100	3.600
12	10.800	5.732	2.639
15	9.600		
18	9.476		

4.2 Comparison of The Colour and Visual Appearance of *Curcuma* spp.

4.2.1 *Curcuma xanthorrhiza*

C. xanthorrhiza extraction produced a yellow to slightly brown-colored extract. *C. xanthorrhiza* rhizomes are known to possess xanthorrhizol and curcuminoids, and the yellow to slightly brownish color of the extract is reflective of these compounds (Figure 4.1) (Kamaruzzaman et al., 2018). The brownish color could reflect other polyphenolic compounds, which have been extracted together with the curcuminoids. In comparison to *C. longa*, *C. xanthorrhiza* extract was slightly lighter in color but possessed a strong yellow pigment. The extract was thicker in consistency relative to *C. long*a, which suggests the existence of other organic compounds such as essential oils responsible for the smell and therapeutic activity of this species (Ali Miftahudin et al., 2011).

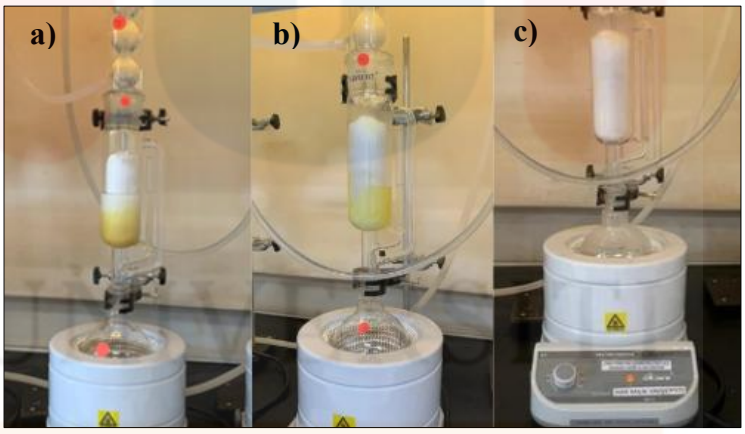

Figure 4.1 a) The mixture of *C. xanthorrhiza s*amples was in a relatively homogeneous state, the beginning to stratify **b)** The middle image shows the progress of the separation process where more noticeable stratification between the phases could be seen **c)** The third image displays the final phase of the extraction where the samples were fully isolated (Nurcholis et al., 2023).

4.2.2 *Curcuma longa*

Curcuma longa is well recognized by its yellow color, which is attributed primarily to its curcuminoids such as curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. During Soxhlet extraction using methanol as the solvent, the solution yielded was orange to deep yellow, showing that the curcuminoids had been isolated successfully (Figure 4.2). This finding concurs with other studies that indicated turmeric extracts' yellowish coloration (Dange, 2023). The bright yellow hue suggests that the curcumin concentration is high, a bioactive compound which is characterized by its antioxidant and anti-inflammatory activity (Figure 4.3) (Jikah & Edo, 2024).

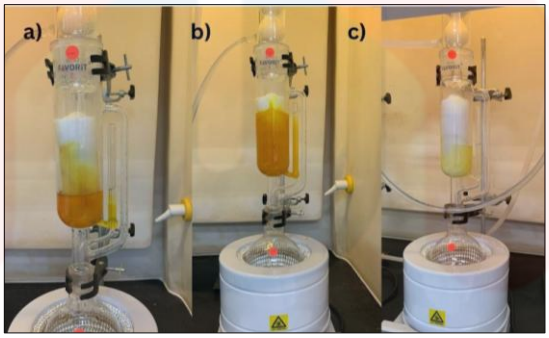

Figure 4.2 a) The initial stage of the extraction of *C. longa* where the separation of the colors started to paper **b)** Intermediate stage where the extraction begun to separate more clearly **c)** The final stage, with clearer separation between the layers, likely indicating that the process was about to complete.

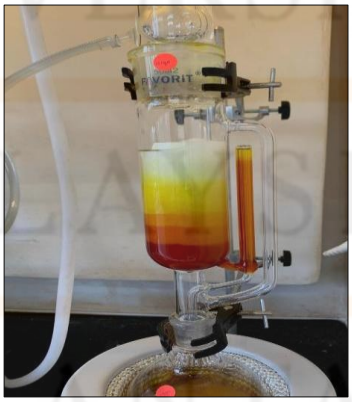

Figure 4.3 The layers of methanol extract of *C. longa* are fully separated, indicating the efficient progress of the extraction (Mungwari et al., 2024).

4.2.3 *Curcuma caesia*

Curcuma caesia or black turmeric produced the darkest extract among the three plants. Curcuma caesia extract was red-brown in color. Camphoraceous smell and unique combination of bioactive constituents like curcuminoids and essential oils are present in the plant (Kress et al., 2022). The dark extract can be attributed to a higher concentration of essential oils and other bioactive constituents of high, aromatic nature.

The dark color of the extract shows that *C. caesia* may have higher volatile compound or secondary metabolite contents, which are likely to be the cause of its medicinal activities like antimicrobial and anti-inflammatory activity (Figure 4.4) (Jayani, 2023). The dark color is consistent with the literature-reported compound profiles where *C. caesia* extracts are volatile oils and curcuminoids (Kamaruzzaman et al., 2018).

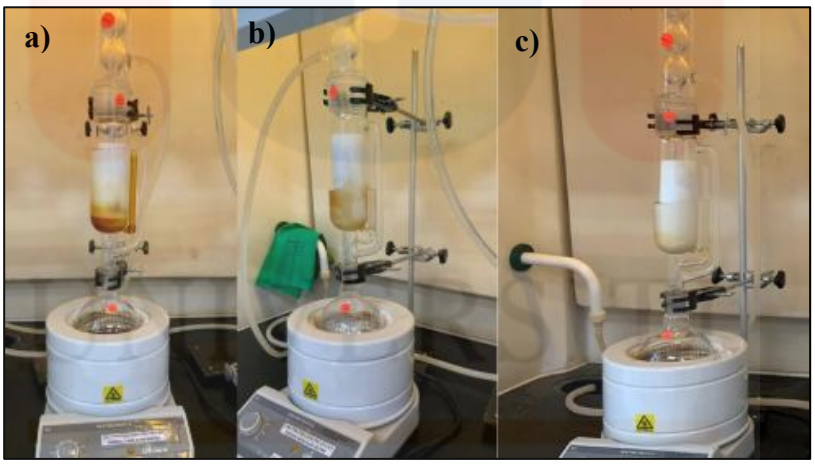

Figure 4.4 a) The beginning of extraction for the samples as thick black color form during the first week of the extraction **b)** The extraction process continued, with the solvent passing through the samples and the color change to greyish black of the solution could be seen **c)** The final stage of extraction where the solution started to become colorless.

4.3 Analysis of Retention Value (Rf) for *Curcuma* spp. via Thin-Layer Chromatography (TLC)

4.3.1 Visualization of The Compound Spots on The TLC Plate

The TLC analysis of *Curcuma longa*, *Curcuma xanthorrhiza*, and *Curcuma caesia* at different concentrations 20, 15, 10, 5, and 0 mg/mL show variations in Retention Factor (Rf) values and spot intensity. The changes in concentration can affect the sharpness and visibility of the spots (Wagner & Bladt, 1996). At higher concentrations which is 20 and 15 mg/mL, more distinct spots are observed due to the higher abundance of active compounds, while lower concentrations show fewer and fainter spots. This indicates that some minor compounds are only detectable at higher concentrations of the extract. (Harborne, 1998). However, excessively concentrated spots can result in spot smearing where the compound spreads beyond the point of application. This may occur due to overloading of the sample on the silica surface, causing the compound to travel unevenly with the mobile phase (Wallace et al., 2012). Smearing also can make it difficult to measure the Rf value accurately and may lead to overlapping of compounds on the TLC plates. This change in intensity might be an indication of more active compounds being deposited and concentrated on the silica plate, producing darker spots (Urbain & Simões-Pires, 2020).

For the analysis of compound spot visibility for *C. xanthorrhiza*, the TLC plate shows moderate to strong spot intensity, especially at higher concentrations such as 20 mg/mL and 15 mg/mL. At 20 mg/mL, several clear and well-defined spots are visible in yellow-brown to orange tones, indicating the presence of multiple compounds. As the concentration decrease to 10 mg/mL and 5 mg/mL, the spots become lighter and less distinct, with some bands becoming faint or nearly invisible (Figure 4.5).

The second spot of 5 mg/mL concentration shows reduction in visibility as the spot barely can be seen compared to other spots on 20, 5 and 10 mg/mL. The compounds responsible for these bands are likely to include xanthorrhizol, a major bioactive compound in *C. xanthorrhiza*, along with other sesquiterpenes and curcuminoid derivatives. These compounds often show moderate pigmentation, which may not appear as intensely colored as the curcumin found in *C. longa*, but still contribute to distinguishable spots.

For the analysis of compound spot visibility for *C. longa*, the visible spots for 20, 15, 10 mg/mL are in high intensity yellow- orange color and most of the spot presence could be observed clearly. The third spot on 20 mg/mL concentration is in the highest intensity of yellowish orange color and reducing to light yellowish brown on 5 mg/mL of extract (Figure 4.6).

This change in intensity may be an indication of more active compounds being deposited and concentrated on the silica plate, producing more darker spots (Li et al, 2019). The compounds that mainly involve can be curcuminoid compounds that are very well-known for their bright yellow-orange pigmentation and since these pigments are more abundant in concentrated extracts, they are more easily visualized in TLC plates (Wang, 2019).

In contrast to other *Curcuma* species, the visible spots recorded at 20, 15, and 10 mg/mL concentrations in the compound spot visibility analysis for *C. caesia* has shown fewer bands and looked as moderate intensity light brown to greyish dots, however at lower concentrations (10 mg/mL and 5 mg/mL), the same spot became noticeably lighter and less defined. It is possible that fewer chemicals were deposited onto the silica surface, leading to lesser pigment expression, as seen by the decrease in intensity and clarity at lower concentrations (Pandey et al., 2025). Besides, the number of spots is decreasing eight to six spots detected on 5 mg/mL of extract (Figure 4.7).

This decrease in spot intensity and clarity at lower concentrations suggests that only a small amount of compound is deposited on the silica surface, making the pigments less visible. This also proves that when the extract is too diluted, it will lead to a lesser number of chemical compounds present to create strong or clear spots on the TLC plate. For example, the compounds likely responsible for the visible spots in *C. caesia* are aromatic turmerones or anthocyanin-related compounds.

These are typical of this species but usually produce duller colors compared to the bright yellow-orange of curcuminoids found in other *Curcuma* species such as *C. longa* and *C. xanthorrhiza*. As a result, the spots from *C. caesia* often appear lighter or more faded. This pattern shows that extract concentration plays a major role in the visualisation of the spots as the higher concentrations contribute to high tendency for the compounds to stick to the plate and produce clearer, darker spots, which makes the compounds easier to detect and analyse (Rafi et al., 2011).

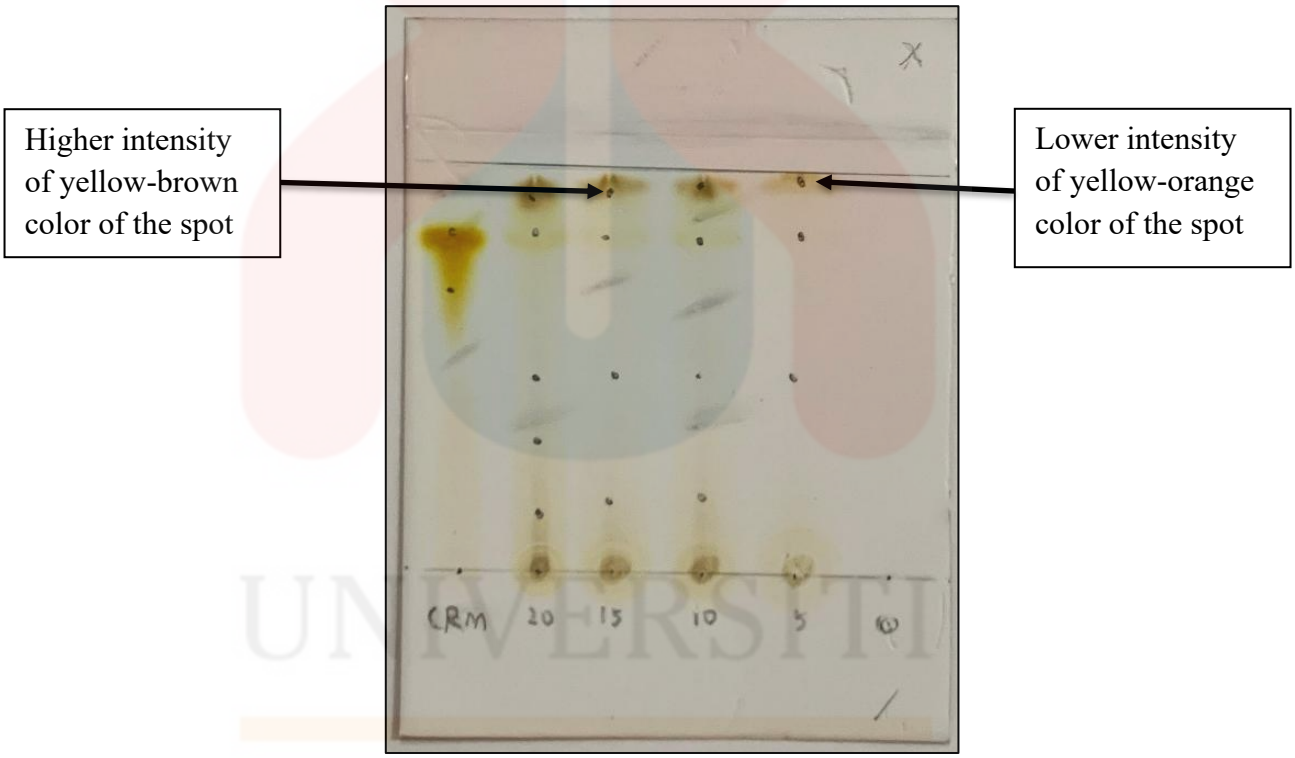

Figure 4.5 TLC profile of *C. xanthorrhiza* extract at different concentrations (20, 15, 10, 5, and 0 mg/mL) compared with curcumin standard (CRM). The yellow bands show the presence of compounds, with intensity reducing at lower concentrations.

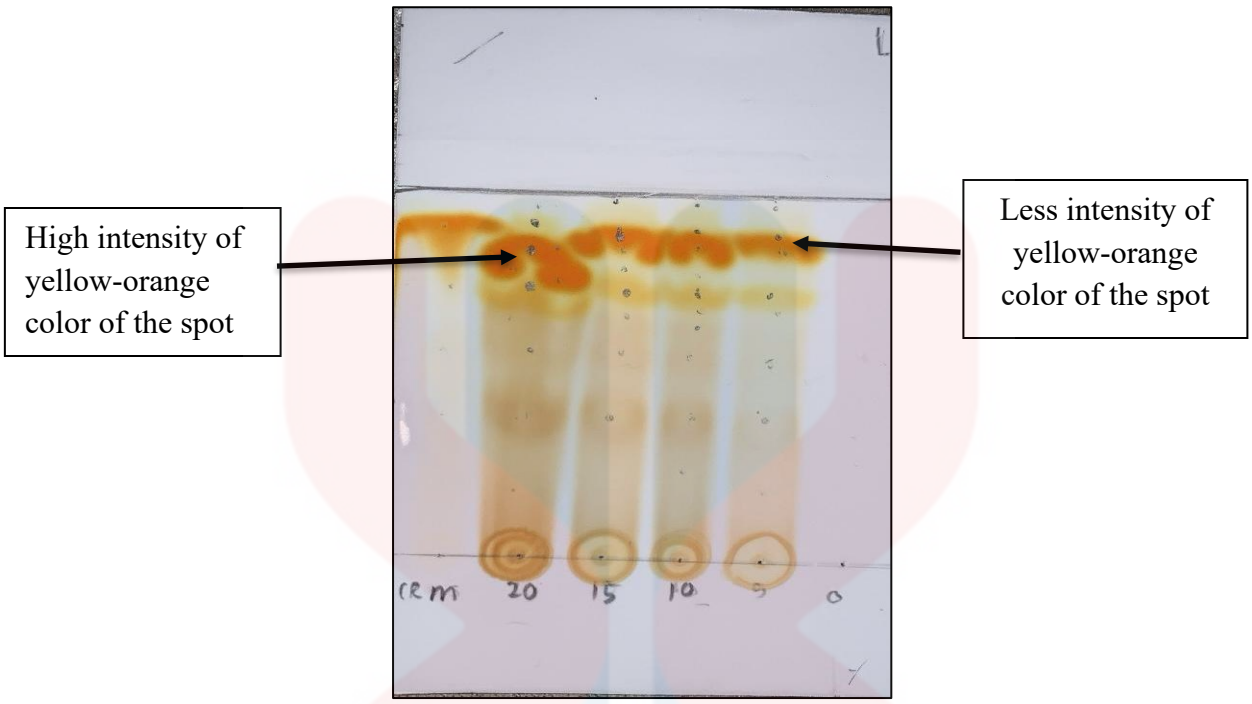

Figure 4.6 The color of the spot on *C. longa* plate for 20, 15, 10 mg/mL of the methanol extract shows distinctly intense yellow-orange spots while the color of the spot was observed at 5 mg/mL concentration slightly lower in intensity.

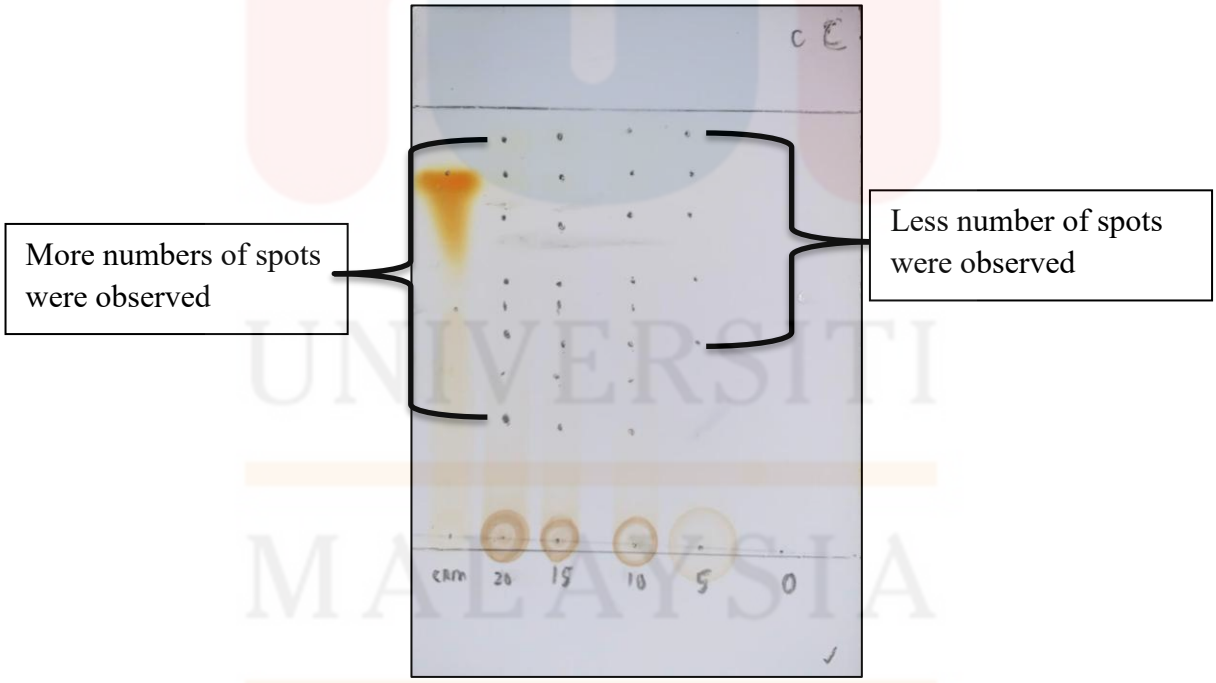

Figure 4.7 The greyish brown color of spots that is visible on *C. caesia* plate, from 20, 15, 10 mg/mL of the methanol extract however the spot color was barely visible at 5 mg/mL concentration.

4.3.2 Retention Factor Analysis of *Curcuma* spp.

Based on a Table 4.3 at 20 mg/mL*, C. xanthorrhiza* exhibited five spots with Rf values of 0.931, 0.862, 0.660, 0.540, and 0.134 (Figure 4.8), The number of spots decreased with concentration at 15 and 10 mg/mL, four spots are recorded maintaining consistent Rf values of 0.939, 0.862, 0.540, and 0.166–0.190, while at 5 mg/mL, three spots were observed with Rf values of 0.939, 0.862, and 0.324. This indicates that the compound exists in the highest concentration which is 20 mg/mL might not exist in any other extract that is diluted in lower concentrations (Figure 4.8). The second spot of most of the extract indicates the presence of the curcumin based on the Rf value calculated with different pigmentation and color visualization due to the different concentrations.

Based on the study conducted by Abdul Rahman (2020) about The Authentication of Java Turmeric (*Curcuma xanthorrhiza*) Using Thin Layer Chromatography and 'H-NMR Based-Metabolite Fingerprinting Coupled with Multivariate Analysis, the presence of curcumin content in this species were in the range of 0.74%- 1.23%. In addition to curcuminoids, *C. xanthorrhiza* also contains xanthorrhizol as one of the main components of antioxidants that produces light-yellow color on the TLC plate**.** This can be proven based on the study conducted on Validated TLC Method for Determination of Curcumin Concentrations in Dissolution Samples Containing *Curcuma longa* Extract by Murti (2015) where the curcumin exist in *C. longa* are the constituent elements of is a naturally occurring phytoconstituent of *C. longa* and other *Curcuma* species in the plant of *C. longa*. Curcumin exists together with the two curcumin derivatives, demethoxycurcumin and bis-demethoxycurcumin (3), which the three compounds are named as curcuminoids.

For the analysis of compound spot visibility for *C. longa*, the visible spots for 20, 15, 10 mg/mL are in high intensity yellow-orange color and most of the spot presence could be observed clearly (Figure 4.9). The third spot on 20 mg/mL concentration is in the highest intensity of yellowish orange color and reduces to light yellowish brown on 5 mg/mL of extract. This change in intensity may be an indication of more active compounds being deposited and concentrated on the silica plate, producing more darker spots (Li et al, 2019). The compounds that mainly involve can be curcuminoid compounds that are very well-known for their bright yellow-orange pigmentation and since these pigments are more abundant in concentrated extracts, they are more easily visualized in TLC plates (Wang, 2019).

The TLC profile of *C. caesia* showed the highest number of bands, with up to eight spots observed at 20 mg/mL, indicating a more chemically diverse composition (Figure 4.10). The most prominent spots occurred at Rf 0.936, 0.838, and 0.804, all of which are close to curcumin but less identical, indicating the presence of curcumin-like compounds such as germacrone and curdione, which have similar polarity and are common in *C. caesia* (Singh et al., 2013). Lower Rf spots such as 0.439, 0.277, and 0.250 may shows the presence of more polar compounds such as β-sesquiphellandrene and xanthorrhizol, which are characteristic of this species and tend to be less attracted due to higher affinity for the silica phase (Chakraborty et al., 2013).

Lower concentrations, 5–10 mg/mL, six consistent bands were still observed, especially at Rf 0.936 and 0.838, indicating that certain compounds are abundant and remain detectable even at reduced concentration of the extract. However, weaker spots such as Rf 0.250 and 0.286 do not appear at lower concentrations, indicating that those constituents are present only in high amounts concentration. This analysis shows that *C. caesia* contains a wider variety of secondary metabolites beyond curcumin, albeit often in lower intensity.

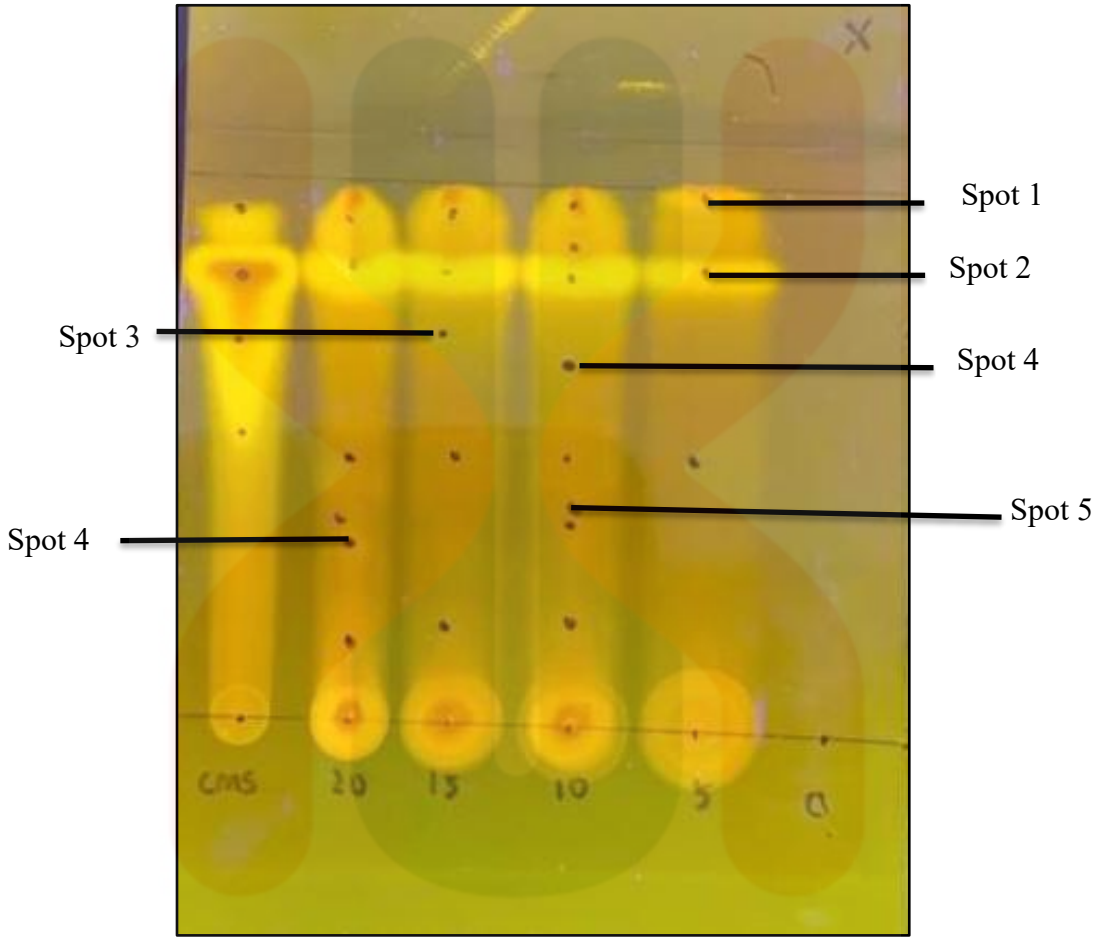

Figure 4.8 TLC plate of *C. xanthorrhiza* extracts under blue light and samples were applied in lanes labeled 20.15,10 and 5 mg/mL and several fluorescent spots with varying Rf values were observed across the lane, indicating the presence of multiple compounds.

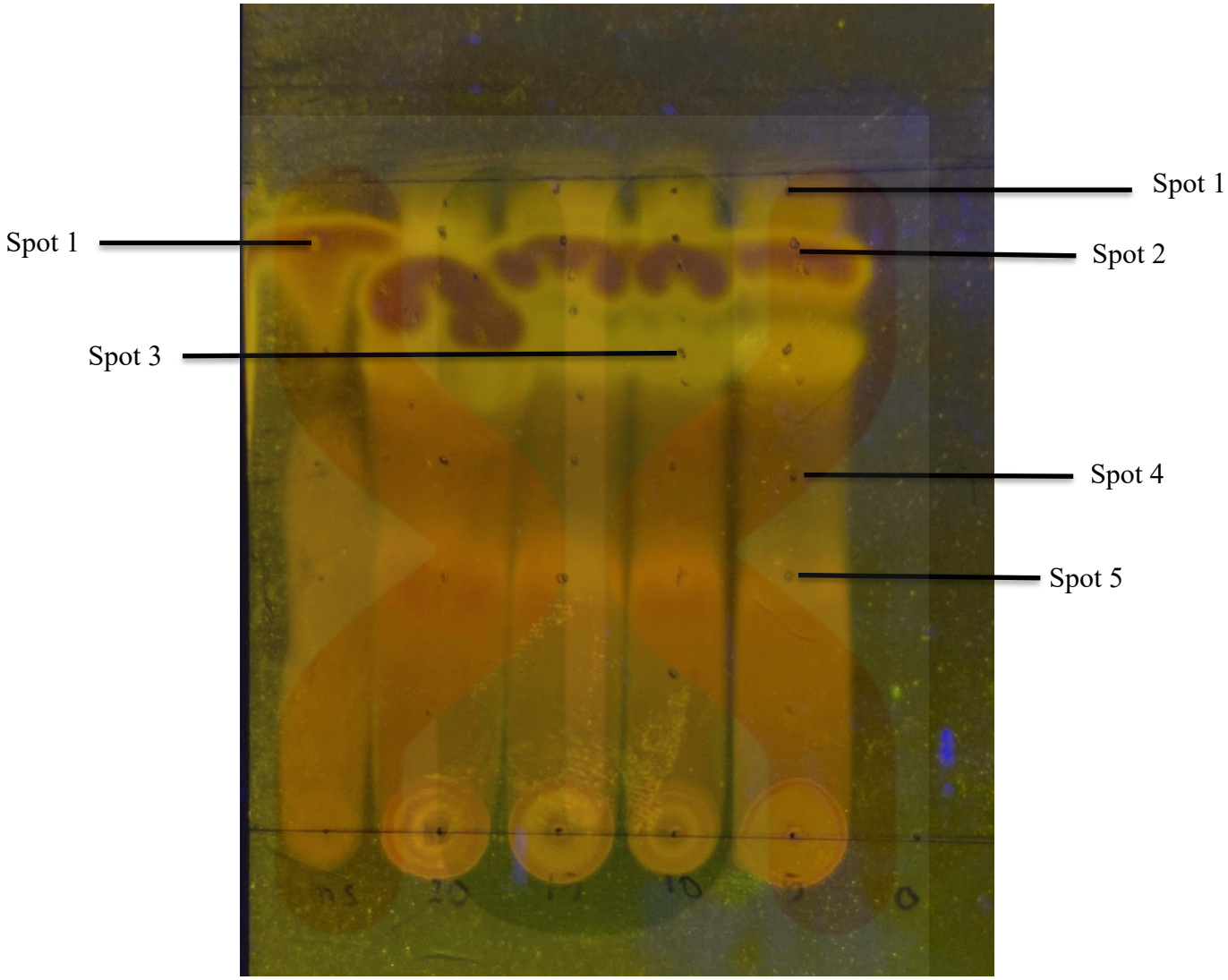

Figure 4.9 TLC plate of *C. longa* under the blue light shows a few visible bands of the compounds on each concentration of the extract where matching spots suggest the presence of curcuminoids (Syarifah et al., 2019)

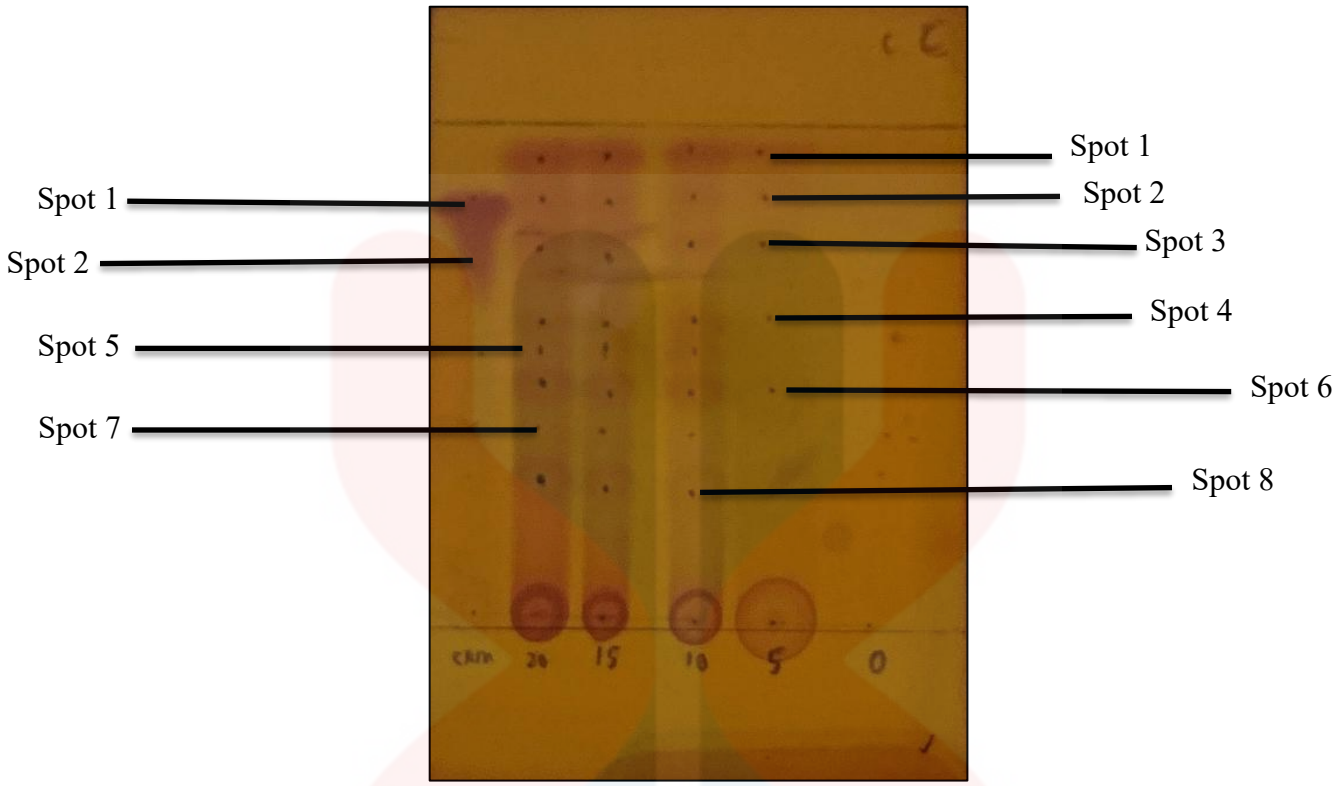

Figure 4.10 TLC plate of *C. caesia* extract under 256 nm shows faint fluorescent spots which limited similarity to the CMR standard, suggesting lower curcuminoid compound.

Table 4.3 Retention factor value of *Curcuma* spp.

Species	Extract (mg/mL)	Retention factor value (Rf) of each spot								
		No. of Spot	Spot 1	Spot 2	Spot 3	Spot 4	Spot 5	Spot 6	Spot 7	Spot 8
CRM	20	2	0.931	0.860						
C. xanthorrhiza	20	5	0.931	0.862	0.660	0.540	0.134			
C. xanthorrhiza	15	4	0.939	0.862	0.540	0.166				
C. xanthorrhiza	10	4	0.939	0.862	0.540	0.190				
C. xanthorrhiza	5	3	0.939	0.862	0.324					
CRM	20	2	0.890	0.780						
C. longa	20	5	0.927	0.862	0.833	0.810	0.637			
C. longa	15	5	0.934	0.868	0.810	0.716	0.654			
C. longa	10	5	0.905	0.860	0.810	0.701	0.192			
C. longa	5	5	0.860	0.810	0.764	0.616	0.912			
CRM	20	2	0.841	0.514						
C. caesia	20	8	0.934	0.838	0.795	0.677	0.567	0.439	0.277	0.250
C. caesia	15	8	0.936	0.838	0.804	0.686	0.572	0.408	0.286	0.213
C. caesia	10	6	0.936	0.838	0.804	0.686	0.572	0.468		
C. caesia	5	6	0.936	0.838	0.804	0.686	0.572	0.448		

Note: These Rf values of each are the average calculation after the replication of TLC.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

As a conclusion, each *Curcuma* species contains distinct compounds at different amounts of concentration. TLC analysis provided different retention factor value (Rf) values for the compound present. These Rf values are crucial for the identification and comparison of phytochemicals, particularly curcumin, a bioactive compound in *Curcuma* species. This study highlights the differences in Rf values across these three species that indicate the diversity of phytochemical compositions. The observed variations in the Rf values can be attributed to differences in the chemical structures and polarities of the secondary metabolites present in each of the species. For example, *C. longa* is known to consist of high concentration of curcuminoids mainly curcumin, desmethoxycurcumin which contribute to its yellow-orange pigment and strong antioxidant activity characteristic (Heger et al., 2014). It also showed that high concentrations of extract contain more dominant compounds such as curcumin than lower concentration of the extract where a few compounds exist that are not visible on the TLC plate. On the other hand, *C. xanthorrhiza* is rich in xanthorrhizol, a sesquiterpenoid compound while *C. caesia* consist of anthocyanin- like plants that are not present in other two *Curcuma* species.

5.2 Recommendations

For the recommendation, the phytochemical differences identified in this study are relevant for bioactivity screening, such as Artemia salina lethality bioassays. Since toxicity is often correlated with the concentration and nature of secondary metabolites, the TLC profiles provide an initial indication of potential bioactive or toxic constituents. For example, fractions rich in curcuminoids or sesquiterpenes can be prioritized for further toxicity testing, enhancing the efficiency of pharmacological or toxicological evaluations (Meyer et al., 1982). TLC characterization of these *Curcuma* species not only reveals their chemical diversity but also offers valuable insights for subsequent HPLC-based compound quantification and bioassay-guided fractionation for toxicity testing (Muppayyanamath et al., 2025).

Based on this study, TLC also can be one of the main components for high-performance thin layer chromatography as the data will complement the objective of HPTLC in statically analysing chemical constituents in herbal plants (Mohammad & Moheman, 2010). During this study, it was observed that the Curcumin Standard Solution (CMS) can effectively assist in spot visualisation. This finding helps save time by eliminating the need to test multiple standard solutions as potential markers. Besides, In TLC, testing with different concentrations of the standard solution allows the identifications of the optimal sample concentration that produces clear and distinct spots on the chromatogram. This step is crucial to avoid wastage during HPTLC analysis, as it ensures that only the most effective concentration is used for accurate quantification and detection, minimizing trial-and-error in the more resource-intensive HPTLC process.

Thus, TLC not only saves time and resources during method development but also ensures that the transition to HPTLC is efficient and scientifically robust, maximizing the reliability of chemical fingerprinting, standardization, and toxicity assessment of herbal and pharmaceutical products (Prasanna et al., 2021).

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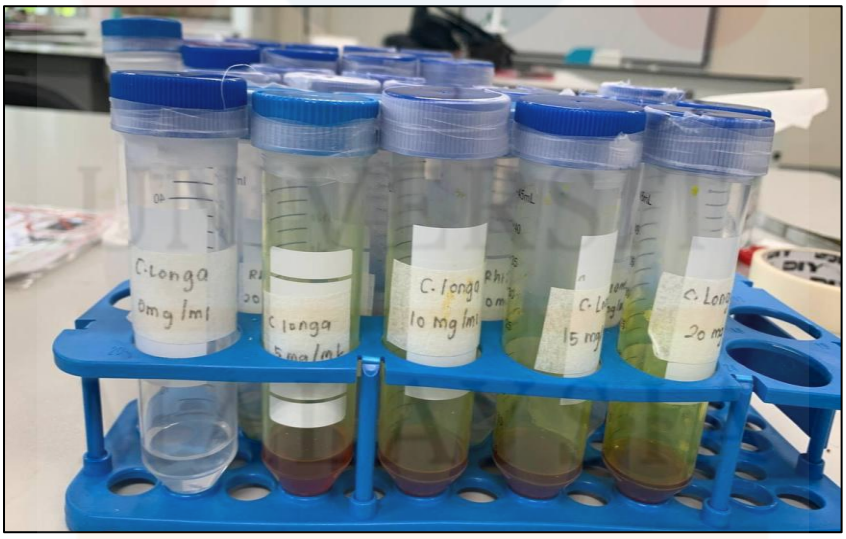
APPENDICES

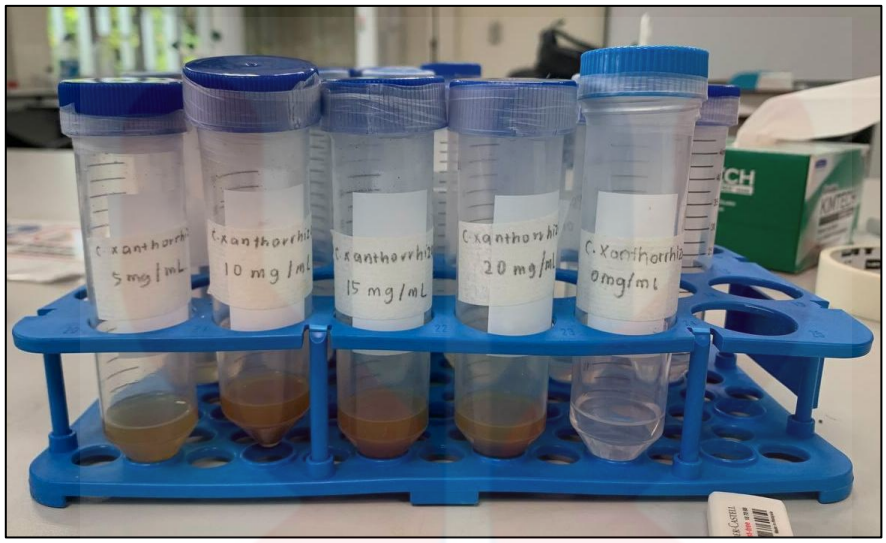
Preparation for stock solution of *Curcuma* spp. Methanol extract.

Dilution of *Curcuma* spp. to create different concentration of methanol extract.

Curcuma longa



Curcuma xanthorrhiza



Curcuma caesia