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UNRAVELLING THE IDENTITY OF BLACK GINGER AND BLACK TURMERIC IN THE MALAYSIAN HERBAL MARKET

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The Zingiberaceae family has long been utilised in Asia for medicinal, culinary, and cosmetic purposes. However, the identification of specific species within this family, such as black ginger and black turmeric, remains challenging in the Malaysian herbal market due to morphological similarities and the lack of standardised identification methods. This study aims to address these challenges by accurately identifying black ginger and black turmeric through comprehensive morphological and chemical analyses. A total of 43 samples of fresh rhizomes and plantlets were collected from nurseries and online platforms across Peninsular Malaysia. The samples were categorized into three groups: Group 1–black ginger (kunyit hitam Thai/halia hitam), Group 2–black turmeric (kunyit hitam India), and Group 3–black turmeric/pink and blue ginger (temu hitam/temu ireng). Morphological characterisation revealed distinct differences in rhizome shape, colour, odour, leaf structure, and inflorescences among the groups. Chemical analyses using HPLC, HPTLC, and FTIR further differentiated the species, with Group 1 displaying a chemical profile consistent with the authenticated *K. parviflora*, marked by the presence of 5,7-dimethoxy flavone. Groups 2 and 3, although morphologically similar, were distinguishable through specific rhizome and leaf characteristics and were chemically similar to the authenticated *C. caesia* and *C. aeruginosa*, respectively. These findings underscore the importance of both morphological and chemical profiling in the accurate identification of these species, which is crucial for ensuring the authenticity and quality of herbal products in the Malaysian market. This study provides a foundation for improved species authentication and contributes to the standardisation of the herbal industry.

Keywords: halia hitam, kunyit hitam, temu hitam, temu ireng, authentication

INTRODUCTION

The Zingiberaceae family had long been used in Asia as traditional medicine, spice and cosmetics, due to their characteristics (colour, taste and odour) and diverse chemical composition. The diversity of the family was mostly concentrated in the South and South–East Asia. In general, the members of Zingiberaceae were small to large, rhizomatous herbs and they were often aromatic. Their leaves were distichously arranged in 2-ranked, forming the pseudostem. Formerly, the Zingiberaceae family had been classified by Burtt & Smith (1972) into 4 tribes, namely Hedychieae, Zingibereae, Alpinieae and Globbeae; based on their morphological features. Both genus, *Curcuma* and *Kaempferia* were placed under the Tribe Hedychieae. In 2002, a new classification of the family using molecular data was proposed by Kress et al. (2002), whereby both *Curcuma* and *Kaempferia* were transferred into the Tribe Zingibereae Meisn., Subfamily Zingiberoideae Haask. Previously, many studies had been conducted on the native Zingiberaceae species in Peninsular Malaysia. Morphologically, without the presence of their inflorescences, members of Zingiberaceae species were difficult to identify down to the genus level due to having similar habit and leaf arrangement. However, the morphology of the non-native ginger species was either not represented or only briefly described and often excluded in the key within the genera in Peninsular Malaysia (Ridley

1924). In addition, the study on Indian *Curcuma* by Leong–Skornickova et al. (2010) showed that some seed producing species could exhibit a wide range of variation, which could lead to misidentification of several species. Meanwhile, a study by Ibrahim et al., 2007 showed that anatomical features of Zingiberaceae leaf could be used in distinguishing the species. Moreover, the study conducted by Chitra and Thoppil (2002) on *Curcuma amada* rhizome transverse section as well as its powder revealed several diagnostic characteristics (i.e. periderm, cork cells, cortex, endodermis, parenchyma cells, vascular bundles, vessels, and starch grains) that could be used to distinguished the plant from adulterants. In addition, Sharma et al. (2017) reported that the combination of standardised parameters, such as macroscopic, microscopic, pharmacognostic, and chemical analysis, could be used in *Curcuma caesia* Roxb.'s authentication. Despite numerous microscopic studies on the Zingiberaceae leaf structure, the rhizome had not been extensively investigated.

From the market survey, the rhizomes (fresh or dried slices) sold associated with black ginger and black turmeric include several vernacular names; i.e. *temu ireng/temu hitam* (black turmeric/pink and blue ginger), *kunyit hitam India/temu hitam* (black turmeric) and *halia hitam/kunyit hitam Thai* (black ginger). Herbal suppliers, manufacturers and consumers often encounter confusion when purchasing the rhizomes using their vernacular names; which were sometimes used interchangeably among the 3 different species. The rhizomes of these species could still be distinguished by colour and smell when fresh. However, they were almost indistinguishable after drying or pulverised. Hence, the species identity could only be solved using chemical analyses. The purpose of this study is to develop a reliable method for identifying black ginger and black turmeric using a combination of morphological and chemical analyses, which has not been previously done in a comprehensive manner. Macroscopic and microscopic examinations are the initial processes for determining the characteristics and degree of purity of medicinal plant materials (Pitakpawasutthi et al. 2019). While morphological and chemical profiling has been used for some species, a comprehensive analysis combining both approaches for black ginger and black turmeric in the Malaysian market is lacking.

Most species had been cultivated in Peninsular Malaysia since the 1920s as a spice or medicine (Ridley 1924). In Peninsular Malaysia, *C. aeruginosa* was used to treat sores (Nik Musa'adah et al. 2017a) while the whole plant decoction had been drunk to relieve muscle pains and strains (Ibrahim et al, 2007). Meanwhile, *temu hitam and kunyit hitam* were present in the Malaysian market in the late 90s. The rhizome of *C. caesia* had been used as a remedy to treat fractures, relief pimples and to alleviate nerve pain (jamu, massage oil or capsule). In addition, the leaves of *C.* caesia were used for bathing after birth (Nik Musa'adah et al. 2017b). Last but not least, the rhizome of *K. parviflora* had been used in Thailand to reduce swelling and treat wounds, diarrhoea and colic (Than & Kyi 2019). Due to the vast benefits of black ginger and black turmeric, this study is crucial for ensuring the authenticity and quality of these herbal products. Currently, there is a lack of reliable identification methods for these plants in the market, which leads to issues like mislabeling or adulteration. The findings from this study can help in standardizing the quality of herbal products in the market, in developing more accurate labeling practices, and increasing consumer safety of traditional herbal products.

Despite the existing studies on the Zingiberaceae family, there is a scarcity of comprehensive information combining morphological, anatomical, and chemical profiles to distinguish these three species in the market. Therefore, this study aims to fill this gap by employing a combination of morphological (macroscopic and microscopic) characterisation and chemical analyses (HPLC, HPTLC, FTIR) to accurately identify black ginger and black turmeric species sold in the Malaysian herbal market.

MATERIALS AND METHODS

Sample collection

Fresh rhizomes and plantlets of black ginger and black turmeric were sought from and procured via nurseries and online platform throughout Peninsular Malaysia. In total, 43 samples consist of fresh rhizomes or plantlets had been

purchased from herbal suppliers, farmers, individual home garden planters and herbal enthusiast. Several prominent suppliers selling these gingers were identified including Daun Hijau Organik (Ulu Sat, Machang, Kelantan), Paya Asli Herbal Valley Qudrifarm (Naka, Kedah), Laman Mek Nab (Parit Buntar, Perak), Khatijah Herbs Traditional (Seri Kembangan, Selangor), Amansahahmad (Jengka, Pahang), Green Durio Trading (Melaka), Urban Ecolife Agrofarm (Gombak, Selangor), Kebun Reyhan/ Herbalwise Holdings (Serdang, Selangor) and Melur.com Nursery (Kota Tinggi, Johor). From the total, the samples were further divided into 3 groups; Group 1) black ginger–kunyit hitam Thai/halia hitam (17 samples), Group 2) black turmeric–kunyit hitam India (15 samples) and Group 3) black turmeric/pink and blue ginger–temu hitam/temu ireng (11 samples).

Morphological characterisation

Young plantlets bought were nurtured in Ethnobotanical Garden, FRIM until reaching maturity state, especially the flowering stage that were crucial for species verification and for rhizome harvesting. The fresh rhizomes harvested from these plantlets were processed, dried and ground into powder and subjected for chemical and microscopic analysis. On the other hand, when samples bought only consist of rhizome, several budding rhizomes were obtained for planting at Ethnobotanical Garden, FRIM. Morphological characteristics of the rhizome (i.e. shape, scent and colour of inner rhizome) procured were examined and recorded. The plantlets grown from the rhizome were used for morphological characterisation and species validation upon maturity.

Morphological characterisation and examination of each vegetative plant parts (i.e. leaf, bladeless leaf sheath, rhizome, root tuber) and inflorescences (when available) were conducted to document the variations exhibit by the samples from various localities and to determine the diagnostic characteristics for species authentication. Species description, key to the genera and species and photos published in botanical manuscripts were referred and comparison of their morphological features with the samples were made.

Microscopic characterisation

Plant microscopy

Fixation, embedding and sectioning were conducted following Johansen (1940) and Sass (1958) with slight modifications. Fresh plant materials were fixed in AA (1:3), consist of 30 % acetic acid and 70 % ethanol. The leaf, sheath and rhizome samples were sectioned using a sliding microtome at 20–30 µm thickness and the tissues (midrib, margin, lamina and petiole) were stained in Safranin and Alcian Green. All plant tissues were then endured the dehydration process using alcohol series 50 %, 70 %, 95 % and 100 % and mounted onto a glass slide using Euparal solution. Finally, photomicrograph of the slides was captured for further analysis. Leaf peeling on both surfaces were also conducted on *Curcuma* species to incorporate additional anatomical features, such as stomata index, number of parallel venations and stomata type.

$$\text{Stomata Index} = \frac{\text{No. of stomata cell (s)}}{\text{No. of stomata cell (s)} + \text{No. of epidemis cell (E)}}$$

Powder microscopy

The finest rhizome powders (10 mg) were sampled and then spread evenly on the slides. 70 % of chloral hydrate was dropped on the slide until the samples were wet. The slide was then covered with the cover slip and observation were carried out under the light microscope with 10–40 × of magnification. Finally, photomicrograph of the slides was captured using the camera adapter mounted on Olympus compound microscope. The images were processed with Top-View Standard software for further analysis.

Chemical fingerprints

HPLC analysis

Instrument: Waters HPLC system consist of a quaternary pump (Waters 600E), an autosampler (Water 717 plus), a PDA detector (Waters 2996 PDA), scanning range from 190–400 nm and a reversed-phase Waters XBridge C-8 column (4.6 i.d. x 250 mm, 5 µm). Software: Empower.

Materials: HPLC grade acetonitrile and purified water.
Sample Preparation: The dried and ground sample, particle size ≤ 500 µm was extracted by sonication with gradient grade solvent in a closed vial for 15 min. The solution was then filtered using 0.45 µm filter cartridge before subjected for analysis.

The chromatograms were generated using a reversed-phase Waters XBridge C-8 column at flow rate of 1.0 mL/min at room temperature and the sample was eluted with a gradient system of 0.1 % aqueous formic acid (A) and acetonitrile (B). The chromatographic elution employed a binary gradient at a flow rate of 1 mL/min with a 10 µL sample injection, commencing at 10% B (0–5 min), transitioning to 40% B (5–50 min) and concluding at 50%–90% B (50–60 min). Detection was performed using the Waters 2996 photodiode array (PDA) detector at the wavelength of 254 nm. Data acquisition and processing were facilitated through the Empower software.

HPTLC analysis

The HPTLC analysis procedures were adopted from Eike & Anne (2007) and Chinese Pharmacopoeia Commission (2009).
Materials: Toluene, chloroform, acetic acid, formic acid, anisaldehyde sulphuric acid.
Sample Preparation: The test solution was prepared by dissolving 0.5 g of the powder (particle size ≤ 500 µm) in 5 mL of methanol, followed by 15 minutes of ultrasonication and filtration, yielding the test solution. A reference solution is generated by dissolving mangiferin (CAS 4773-96-0) in methanol to a concentration of 1 mg/mL.
Chromatographic analysis employs an HPTLC silica gel pre-coated plate 60 F254 (10 × 10 cm) as the stationary phase, with a mobile phase comprising of toluene, chloroform, acetic acid in the ratio of 5:4:1:0.2 (20 mL total volume). Meanwhile, the test samples and reference solutions (2 µL each) were applied separately as 6 mm bands onto the plate, spaced 5 mm apart. The plate underwent vertical development for 7.0 cm in a twin trough chamber (10 cm × 10 cm), following equilibration with the mobile phase for 15 minutes. Subsequently, the plate was immersed in the anisaldehyde sulphuric acid and heated at 105°C until zones become visible. The plate was then examined under daylight and ultraviolet light at the wavelengths of 254 nm and 366 nm, respectively.

Multi-steps infrared macro-fingerprinting

Instrument: Spectrum 100 Series Fourier transform-infrared spectrometer: (Perkin Elmer, CA, USA), equipped with a mid-infrared deuterated triglycine sulphate (DTGS) detector. Scan range: from 450–4000 cm-1 with a resolution of 4 cm-1. Spectra were obtained from the accumulation of 16 scans. Temperature controller (4000 seriesTM High Stability Temperature Controller, Specac, Ltd): 50–120 °C.
Software: Softdoc developed by Tsinghua University (Beijing, China).
Materials: Spectrophotometric grade potassium bromide (KBr). Sample Preparation: The dried, powdered material were blended with potassium bromide (KBr) powder, in the ratio of 2 mg to100 mg. The mixture was transferred into a mould and was hydraulic pressed to form a 13 mm diameter KBr disc. Each sample were analysed in triplicates.
One and Two-dimensional Infrared Spectral Analysis: 1D-IR spectra were recorded from a total of 16 scans in the 4000–450 cm-1 range with a resolution of 4 cm-1. The second derivative spectra were obtained by means of Savitzky-Golay filter through 13-point smoothing. Savitzky-Golay smoothing aims for minimum distortion by least squares fitting a cubic polynomial. For the measurement of 2D-IR spectra, each sample disc was placed into the sample pool connected with a temperature controller. Dynamic spectra were collected at different temperatures ranging from 50 to 120 °C at an interval of 10 °C.

RESULTS

Morphological and microscopic characterisation

In general, all samples were rhizomatous, herbaceous plants. The main rhizomes were ovoid or globose in shape, aromatic and had thick, cylindrical roots. Meanwhile, the root tubers were obovoid, ellipsoid or spindle-shaped, the outer surface was white or pale

brown, inner white. The leaf blade was broad ovate or elliptic–oblong with asymmetry base, subcoriaceous and had subentire margin. In addition, the petiole adaxial was 'U' grooved with subrounded abaxial, and had subglabrous or hairy surface; the ligule was triangular and caducous.

From the petiole transverse section (TS), the abaxial outline for all samples was U-shaped and had open, U-shaped main vascular strand with the presence of sclerenchyma cells (Figure 1 a, b & c). In addition, medullary bundles, air canals/lacuna, mucilage and oil cells, and prism calcium oxalate crystals were also present on both petiole and midrib TS (Figure 1 d, e & f). Moreover, lamina TS exhibit one layer of epidermis with thin cuticle at both surfaces and the presence of 1–2 hypodermal layer (Figure 2 a, c & e). Furthermore, in general, the margin TS of all samples were tapered (Figure 2 b, d & f). The root tuber TS had circular outline (Figure 3 a & c) while the sheath TS had 'U' shaped outline on both adaxial and abaxial (Figure 3 b & d). Meanwhile, the powdered rhizome examined revealed several microscopic structures such as starch granules, curcumin cells and oil cells (Figure 4).

Chemical fingerprints

HPLC and HPTLC analysis

The HPLC and HPTLC profiles for the three sample groups, Group 1 (Figure 6c, Fig. 7a), Group 2 (Figure 6f, Fig. 7c) and Group 3 (Figure 6i, Fig. 7b) were generated respectively. One representative from each sample group that best fitting the rhizome and plant morphology descriptions was chosen as authentic species. The group profiles generated was compared with the authenticated species of *K. parviflora* (Figure 6b, Figure 7a, Track 2), *C. caesia* (Figure 6e, Figure 7c, Track 2) and *C. aeruginosa* (Figure 6h, Figure 7b, Track 2) respectively. The profiles provide significant insights into their phytochemical compositions and allows for effective differentiation of these species, as indicated by the selected UV spectra peaks (Figure 6d, g and j). Meanwhile, 5,7-dimethoxyflavone was used as reference (Figure 6a & Figure 7 Track 1) to distinguish *K. parviflora*. All samples of Group 1 including authenticated *K. parviflora* revealed the presence of this chemical marker as in Figure 7 a (yellow bands, Rf 0.62) and Figure 6b and c (red peak).

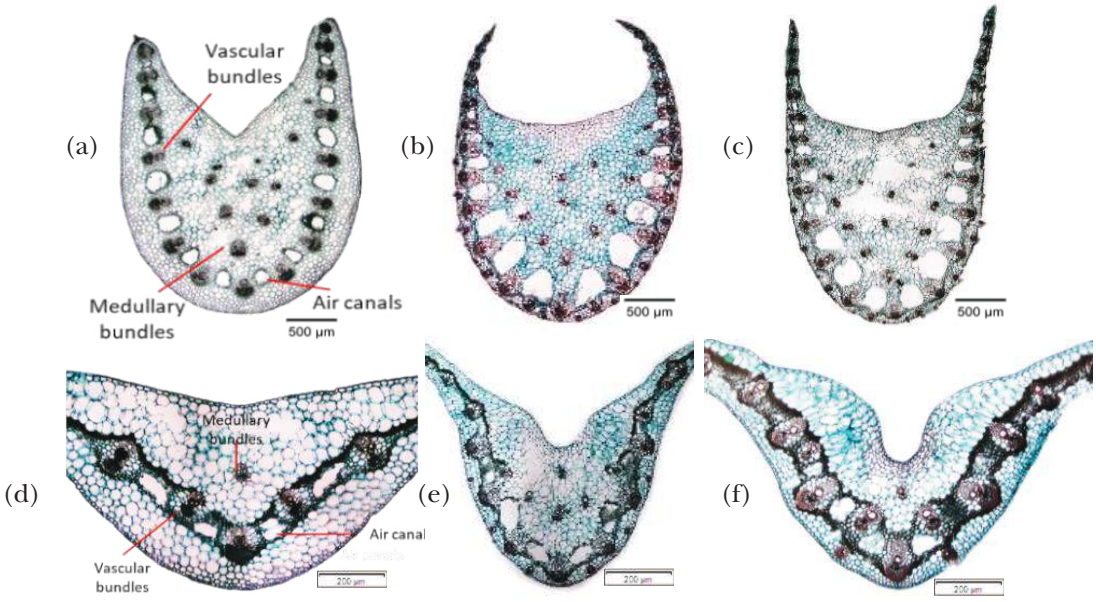

Figure 1 Leaf transverse section. Petiole of a) Group 1, b) Group 2 and c) Group 3. Midrib of d) Group 1, e) Group 2 and f) Group 3

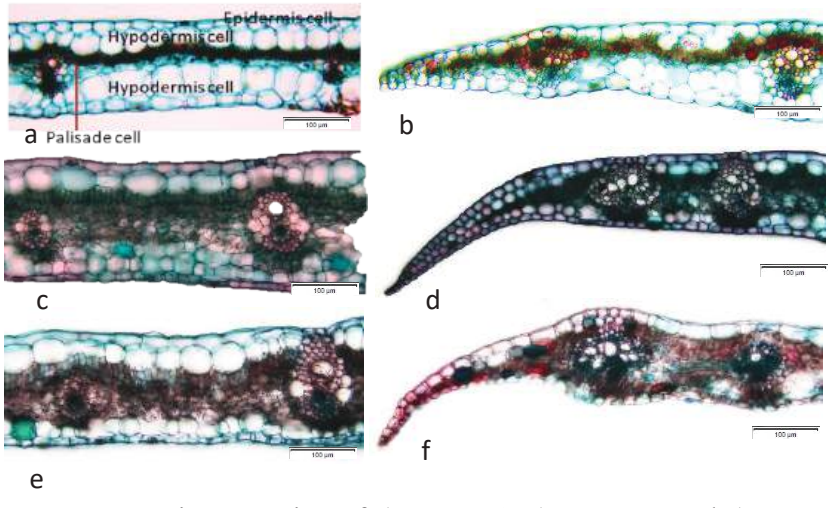

Figure 2 Leaf transverse section. Lamina of a) Group 1, c) Group 2 and e) Group 3. Margin of b) Group 1, d) Group 2 and f) Group 3

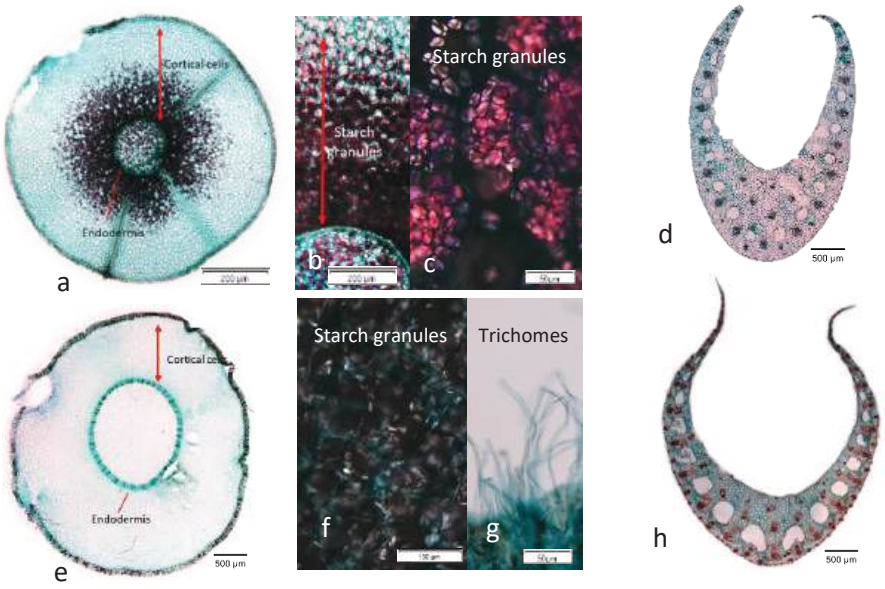

Figure 3 Root tuber transverse section (TS) and microscopic features observed in a) Group 1 and e) Group 2 and 3. Sheath TS of d) Group 1 and h) Group 2 and 3

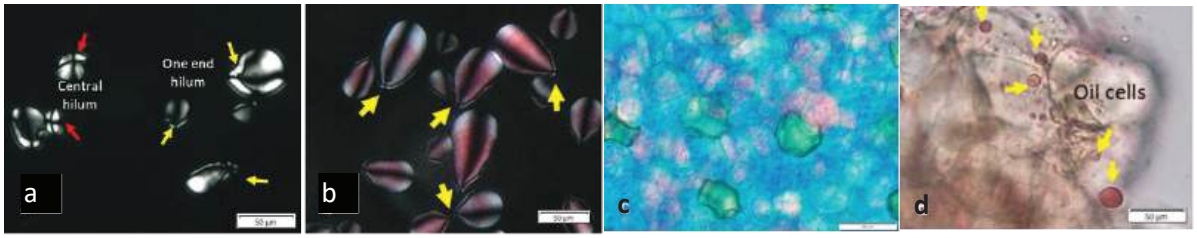

Figure 4 Powder microscopy of rhizome revealed presence of a) Starch granules (central and one end hilum), b) Starch granules (one end hilum), c) Curcumin cells and d) Oil cells

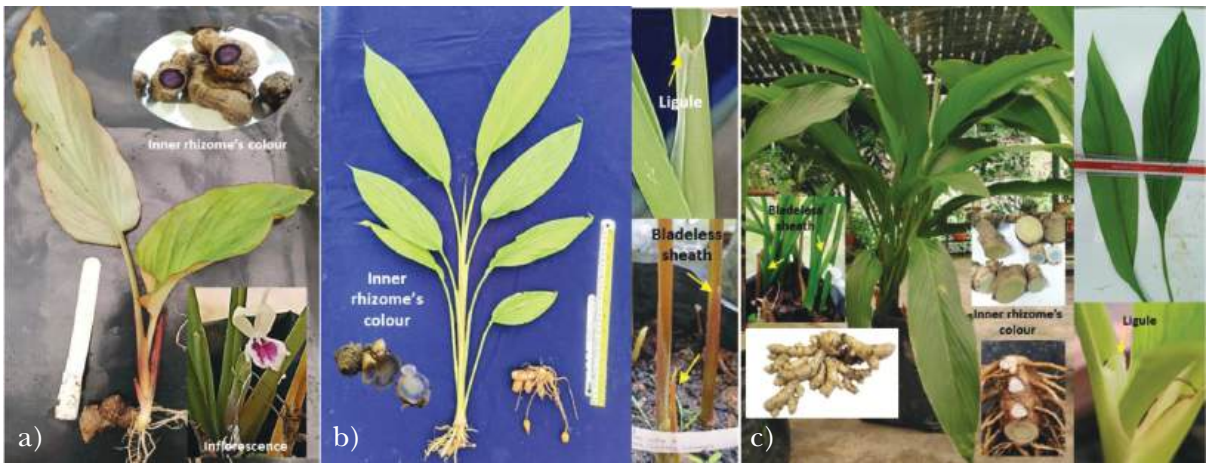

Figure 5 Plant habit and diagnostic characteristics of: a) Group 1–*Kaempferia parviflora* Wall. ex Baker, b) Group 2–*Curcuma caesia* Roxb. and c) Group 3–*Curcuma aeruginosa* Roxb

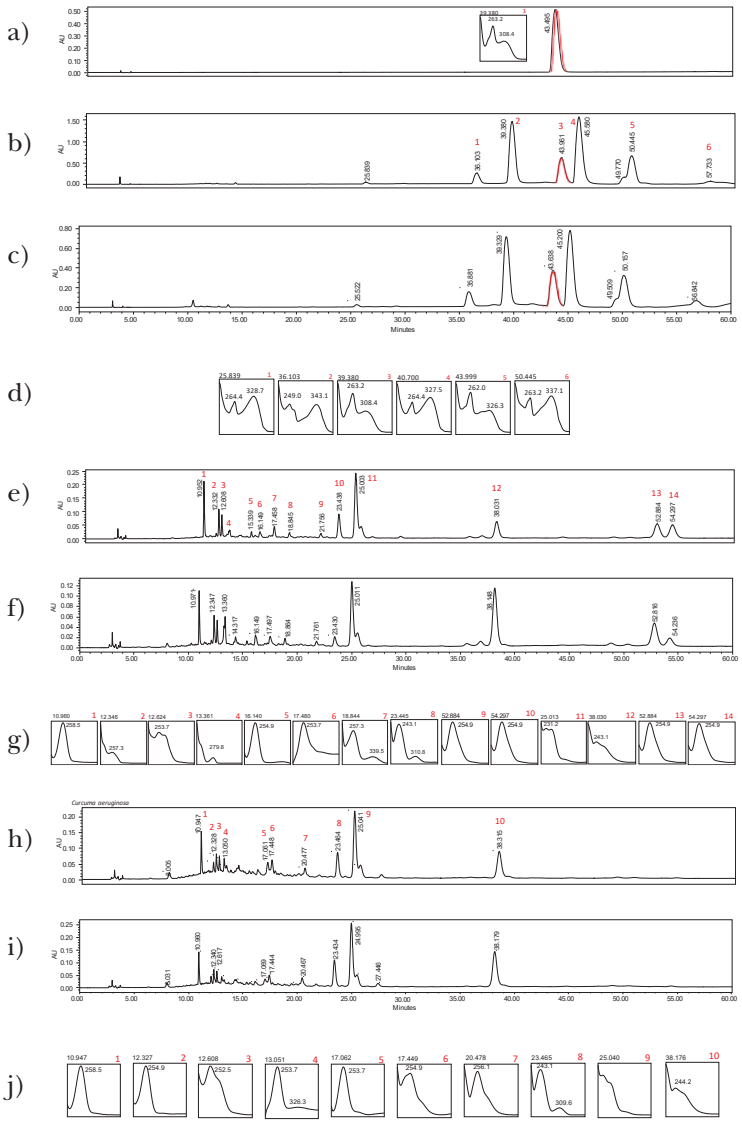

Figure 6 HPLC chromatogram for a) 5,7-dimethoxyflavone, b) authenticated *K. parviflora*, c) Group 1, e) authenticated *C. caesia*, f) Group 2, h) authenticated *C. aeruginosa* and i) Group 3; UV spectra for d) *K. parviflora*, g) *C. caesia* and j) *C. aeruginosa* chromatograms respectively

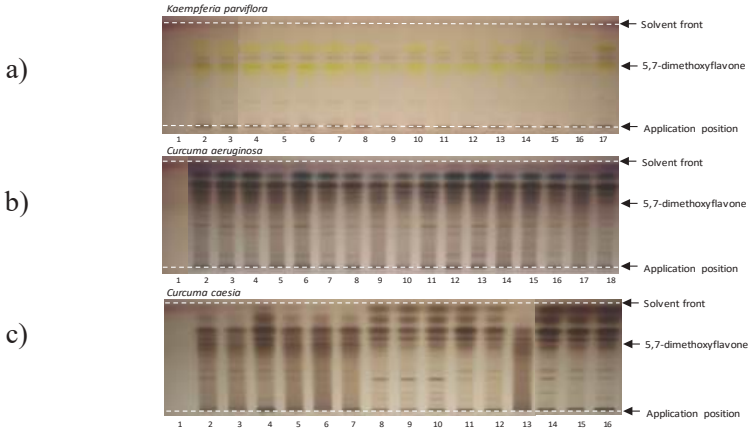

Figure 7 a) Track 1, 5,7-dimethoxyflavone; track 2 authenticated *K. parviflora*; track 3–17, Group 1 from various locations; b) Track 1, 5,7-dimethoxyflavone; track 2 authenticated *C. aeruginosa*; track 3–18, Group 3 from various locations; c) Track 1, 5,7-dimethoxyflavone; track 2 authenticated *C. caesia*; track 3–16, Group 2 from various locations

Multi-steps Infrared Macro-fingerprinting

1D-IR and second derivatives spectral analysis

The spectral profiles for the 3 groups in the range of 4000–450 cm^{-1} was presented on Figure 8a. The O-H (hydroxyl group), the asymmetric C-H and aromatic ring skeleton stretching vibrations were exhibited at the range 3500–3200 cm^{-1}, 2927–2932 cm^{-1} and at 1600 cm^{-1} respectively. Notable differences were observed in the peak positions and intensities at the range of 1950–873 cm^{-1} (Figure 8b). The unique absorption peak at 1352 cm^{-1}, attributed to the C=C bond, commonly found in the flavones group easily distinguished Group 1 (*K. parviflora*) from the rest. In addition, the presence of 2 peaks at 1375 cm^{-1} and 1324 cm^{-1} successfully delimit Group 3 (*C. aeruginosa*). On contrary, Group 2 (*Curcuma caesia*) could be distinguished by the absence of these 3 peaks. Moreover, second derivatives spectral (Figure 8c) further resolved overlapped absorption peaks, highlighting dissimilarities, especially in the region 1800–1300 cm^{-1}. Group 1 (*K. parviflora*) showed strong, sharp absorption peaks at 1605 cm^{-1} and 1492 cm^{-1}. Meanwhile, Group 3 (*C. aeruginosa*) could be distinguished by the present of 1489 cm^{-1} and 1324 cm^{-1} peaks which were absent in Group 2 (*C. caesia*).

Two-dimensional (2D) IR spectral analysis

2D-IR Spectroscopy was effective in distinguishing the three groups based on unique spectral features and cross peaks. Group 1 (*K. parviflora*) revealed disintive auto-peaks and strong peaks at specific frequencies (Figure 8d A). Meanwhile, Group 2 (*C. caesia*) displayed unique carbonyl vibration peak and distinct cross peaks (Figure 8d B). In addition, Group 3 (*C. aeruginosa*) had similar cross-peaks as *C. caesia* but distinguishable through the present of additional peaks (Figure 8d C).

DISCUSSION

Morphological and microscopic characterisation The morphology of Group 1–Black Ginger observed in this study match the plant descriptions of *Kaempferia parviflora* Wall. ex Baker (Sirirugsa 1992). In addition, the species was also confirmed as *K. parviflora* using the key and inflorescence characteristics provided. Moreover, the variations on the rhizome colour (purple to dark purple), petiole length (short to long) and leaf margin colour (red or faint red) recorded were similar as the findings by Labrooy et al. (2018), with slight differences whereby samples with long petiole, green or faint red margin might also exhibit dark purple inner rhizome colour. Several microscopic findings from our study such as petiole outline,

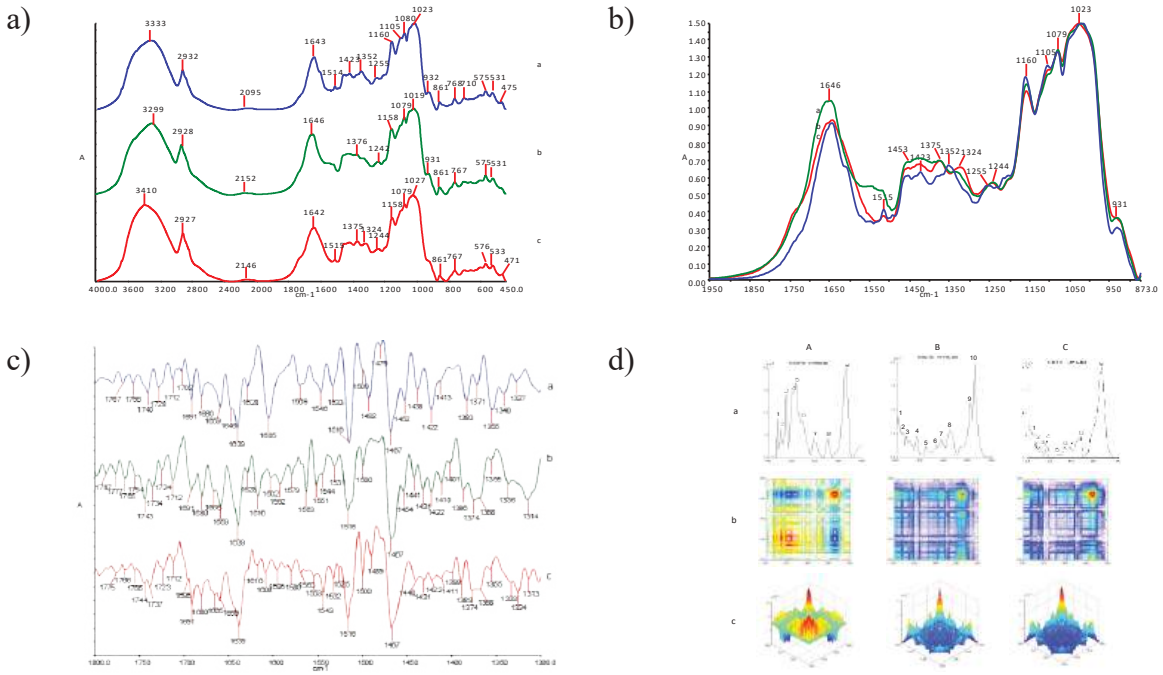

Figure 8 a) 1D-IR spectra, b) 1D-IR spectra in the range of 1950-873 cm^{-1}, c) secondary derivatives IR spectra in the range of 1800-1300 cm^{-1}, d) 2D-IR correlation spectra [a auto-peaks plots, b contour plots, c mesh plots] for Group 1–*K. parviflora* (blue, A), Group 2–*C. caesia* (green, B) and Group 3–*C. aeruginosa* (red, C)

crescent shaped vascular bundles, oxalate crystals and the presence of starch and vessels on the rhizome powder coincide with the study conducted by Than et al. (2019) and Ibrahim et al. (2007).

The morphology of Group 2–Black turmeric and Group 3–Black Turmeric/Pink & Blue ginger examined in this study match the morphological descriptions of *Curcuma* (Sirirugsa et al. 2007, Mangaly & Sabu 1993), while the leaf (shape, colour) and rhizome (shape, inner colour) of *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. were as described by Roxburgh (1810) respectively. Meanwhile, the vegetative morphological characteristics of Group 3 (i.e. bladeless sheath colour-green, ligule) were in accordance with the diagnostic characteristics of *C. aeruginosa* reported by Leong-Skornickova (2003). On the other hand, the microscopic findings showed that both *C. caesia* and *C. aeruginosa* were very similar and could only be differentiated using the stomata index and number of parallel venations on the adaxial epidermis cells (Figure 9). The stomata index of Group 2, *C. caesia* was 9.2, relatively more than Group 3, *C. aeruginosa*, at 7.8, similar with the study conducted by Dutta (2017). This microscopic feature proved to be useful in distinguishing these two closely *Curcuma* species especially when both plants devoid of inflorescence.

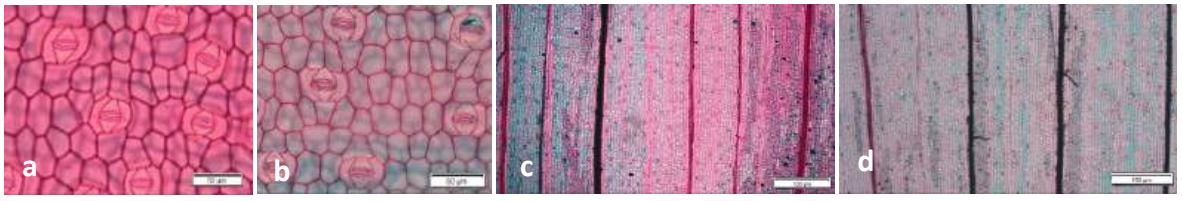

Figure 9 Tetracytic stomata and adaxial venation of *C. caesia* (a & c) and *C. aeruginosa* (b & d)

Table 1 Diagnostic morphological and microscopic characteristics of Group 1, Group 2 and Group 3 in Peninsular Malaysia

Characteristics	Group 1 (*Kaempferia parviflora* Wall. ex Baker)	Group 2 (*Curcuma caesia* Roxb)	Group 3 (*Curcuma aeruginosa* Roxb)
Habit	Small plant with 3–5 erect leaves, up to 55 cm tall	Medium plant, leaf distichously arranged into erect pseudostem, reaching 1 m tall	Medium plant, leaf distichously arranged into erect pseudostem, reaching 1.5 m tall
Rhizome	Main: Small, ovoid–oblongoid, growing laterally, not branching; inner colour purple to dark purple (Figure 5a inset rhizome), emitting soft, jasmine scent.	Main: Big, ovoid–globose, apex protruding above the ground, branching laterally below the ground; inner colour similar with branched.	Main: Big, ovoid–globose, apex protruding above the ground, branching laterally below the ground; inner colour purplish outer ring and yellowish core
	Lateral/branched: Nil	Lateral/branched: Compact, 1–2 times branched, short cylindrical or ovoid; inner (both main and branched) colour pale blue–purple, with yellowish core or light purple–blusih purple, more intense towards the edge (Figure 5b rhizome); occasionally bluish purple, emitting strong, aromatic to slightly pungent scent	Lateral / branched: Condensed; profusely 2–3 times branched, short–long cylindrical or ovoid; inner colour pale blue–blue or very pale blue–yellow (Figure 5c inset rhizome), emitting faint, sweet to aromatic scent.
	Root tubers few	Root tubers lacking or few	Root tubers many
Rhizome Powder Starch granule	Versiform shaped; centre and one end hilum (Figure 4a)	Cylindrical to phseoliform; one end hilum (Figure 4b)	Cylindrical to phseoliform; one end hilum (Figure 4b)
Root tuber microscopy Trichome	Absent	Present (Figure 3 g)	Present (Figure 3 g)
Leaf	Blade broad ovate, ovate or ovate–lanceolate	Blade elliptic–oblong or lanceolate	Blade elliptic–oblong or lanceolate
	Apex acute or subacute, Base subcordate.	Apex abruptly acuminate, Base cuneate.	Apex acuminate, occasionally subacute, Base cuneate, decurrent.
	Margin undulate, green or red.	Margin hyaline or white.	Margin hyaline or white.
	Adaxial–green, glabrous,	Adaxial–green with 1/2–3/4 purple patch of leaf length, subglabrous,	Adaxial–green with 1/3–1/2 purple patch at leaf distal, fading upon maturity, subglabrous,

continued

Table 1 Continued

Characteristics	Group 1 (*Kaempferia parviflora* Wall. ex Baker)	Group 2 (*Curcuma caesia* Roxb)	Group 3 (*Curcuma aeruginosa* Roxb)
	Abaxial–paler green, hirsute hairy.	Abaxial–paler green with brown patch traces from leaf adaxial, subglabrous to pubescent.	Abaxial–paler green, pubescent or subglabrous.
	Petiole short–long Ligule triangular, 1 mm, margin glabrous, apex subacute	Ligule triangular, 2–4 mm, margin hairy or subglabrous, apex acuminate, occasionally tufted (Figure 5b ligule)	Ligule triangular or obscurely bilobed, 1 mm, margin glabrous–subglabrous, apex obtuse. (Figure 5c ligule)
Leaf microscopy			
Petiole outline	Adaxial– 'V' shaped (Fig. 1 a)	Adaxial – 'U' shaped (Figure 1 b)	Adaxial – 'U' shaped (Figure 1 c)
Midrib outline	Adaxial– concave; Abaxial– arc-shaped (Figure 1 d)	Adaxial and abaxial – 'V' shaped (Figure 1 e)	Adaxial and abaxial – 'V' shaped (Figure 1 f)
Margin	Straight (Figure 2 b)	Pointing downward at varying degree (Figure 2 d)	Pointing downward at varying degree (Figure 2 f)
Bladeless leaf sheath colour	Red to reddish green	Reddish green or purplish/reddish brown (Figure 5b bladeless sheath)	Green (Figure 5c inset bladeless sheath)
Inflorescence	Enclosed by 2 innermost leaf sheaths or bladeless leaf sheath; Flower white, staminodes oblong, labellum with purple patch, apex shallowly divided or emarginate, anther-crest entire (Figure 5a inset Inflorescence)	None observed	None observed

Chemical fingerprints

The chemical fingerprinting of *Kaempferia parviflora, Curcuma caesia,* and *Curcuma aeruginosa* using HPLC, HPTLC, and IR spectroscopy provided robust methods for distinguishing these species. *K. parviflora's* HPLC profile is dominated by polymethoxyflavones, with significant peaks such as 5,7-dimethoxyflavone, consistent with previous studies (Yenjai et al. 2004, Ngoc Khanh Pham et al. 2021). In contrast, *C. caesia* and *C. aeruginosa* are primarily characterised by their curcuminoid content, with *C. caesia* showing distinct peaks for curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Jayaprakasha et al. 2002). *C. aeruginosa* displayed similar curcuminoid peaks but with varying intensities and retention times, aiding in differentiation. The HPTLC profiles complement these findings, with *K. parviflora* showing multiple bands for polymethoxyflavones, notably 5,7-dimethoxyflavone, aligned with the HPLC results. Similarly, *C. caesia* and *C. aeruginosa* displayed bands related to curcuminoids and essential oils, with variations in intensity and Rf values that reinforce the HPLC data (Jayaprakasha et al. 2002, Li et al. 2011).

In addition to HPLC and HPTLC analyses, the incorporation of multi-step infrared (IR) spectroscopy further enhanced our approach to distinguishing *Kaempferia parviflora, Curcuma caesia,* and *Curcuma aeruginosa*. The 1D-IR spectra revealed distinct variations among the species, with more pronounced features in the second derivative spectra, reflecting the unique chemical profiles of each species. These spectral differences underscored the diverse chemical constituents present in the samples, influenced by their species. Additionally, 2D-correlation analysis provided deeper insights into the similarities and differences among the species under thermal perturbation, enriching our understanding of their chemical composition and structural characteristics.

The combination of HPLC, HPTLC, and IR spectroscopy offers a holistic approach for analysing and differentiating these species. Each technique contributes unique insights into the phytochemical compositions of these medicinal plants, enabling robust identification and authentication. This integrated approach not only enhances the accuracy and reliability of phytochemical analysis but also serves as a valuable tool for quality control in the herbal industry. By leveraging the strengths of HPLC and HPTLC for detailed chemical fingerprinting with IR spectroscopy's ability to detect functional groups and macroscopic differences, we have addressed the initial research gap. This ensures a thorough and reliable species identification process, providing a more comprehensive understanding of the phytochemical diversity among *K. parviflora*, *C. caesia*, and *C. aeruginosa*.

CONCLUSION

In conclusion, this study effectively identified *Kaempferia parviflora* Wall. ex Baker, *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. in the Malaysian herbal market using a combination of morphological, microscopic, and chemical analyses. Key diagnostic features and chemical fingerprints were established, enhancing the accuracy of species authentication. These findings provide valuable tools for quality control in the herbal industry, ensuring the accurate identification of medicinal plants and contributing to the safety and efficacy of herbal products. Future research could further explore the therapeutic potential of the identified compounds.

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