Abstract

*Cymbopogon nardus* (*C. nardus*) or fragrance lemongrass is a member of *Gramineae* family which is well known for its citronella oil content that is commonly used as a natural fragrant oil, in insect repellents, as well as in beauty, household and perfumery products. *C. nardus* is one of the unique herbs that has been widely discussed in Malay Medical Manuscript and has been consumed by Malay practitioner as it possesses various anti biological activities such as anti fungal, anti bacteria and anti-inflammatory. In traditional system of medicine among Malay traditional people, *C. nardus* has been used for the treatment of fever, muscle pain, and many kinds of illness. Various uses shown by this herb and the tendency of high application of the herb among Malay society has led us to study *C. nardus* according to the scientific perspective. Therefore, the objectives of this study are to identify the morphology of *C. nardus* and its application from Malay traditional’s perspectives, as well as to determine the active compounds present in the herb through scientific findings. Two different parts of leaves and stems of *C. nardus* were extracted using steam distillation method for 2 hours. The chemical composition of citronella oil was determined by Gas Chromatography-Mass Spectrometry (GC-MS).
amount of 0.55 % and 0.024 % of citronella oil were extracted from leaves and stems parts, respectively. The main compositions of oil extracted from the leaves part are citronellol (41.4 %), methyleugenol (80.2 %), citronellal (39.7 %), D- limonene (40 %) and geraniol (36.5 %). Meanwhile, the extraction from the stems composed of α-Himachalene (45.34%), Citronellol acetate (48.2 %), cis-Geraniol (57.8 %) and endo-Borneol (26.2 %). Thus, it can be concluded that C. nardus leaves extraction yield higher citronella oil content as compared to stems and consist of different main chemical compositions between the two parts. Apart from that, the results of this study also prove that the phytochemical content found in C. nardus has anti-fungal properties, capable of treating skin and sinus diseases, as being applied by Malay traditional practitioners in 19M century. This suggests the wisdom of the Malay community in adapting their life skills to the nature as they able to integrate all parts of fragrance lemongrass to be used as medicines.

**Keywords:** Cymbopogon nardus; Stems; Leaves; Malay Medical Manuscript

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**Abstrak**

Cymbopogon nardus (C. nardus) atau serai wangi adalah ahli keluarga Gramineae yang terkenal dengan kandungan minyak citronella yang biasa digunakan sebagai minyak wangi semula jadi, penghalau serangga, produk kecantikan dan produk peralatan rumah serta produk wangi. C. nardus adalah salah satu ramuan unik yang telah dibincangkan secara meluas dalam Manuskrip Perubatan Melayu dan telah digunakan oleh pengamal Melayu kerana ia mempunyai pelbagai aktiviti anti biologi seperti anti fungus, anti bakteria dan anti-radang. Dalam sistem perubatan tradisional dalam kalangan rakyat tradisional Melayu, C. nardus telah digunakan untuk merawat pelbagai jenis penyakit antaranya demam, sakit kepala, sinus, typhoid, hernia dan lain-lain. Pelbagai kegunaan yang ditunjukkan oleh herba ini dan kecenderungan penggunaan herba tinggi dalam kalangan masyarakat Melayu telah membawa kami untuk mengkaji C. nardus mengikut perspektif saintifik. Oleh itu, objektif kajian ini adalah untuk mengenali pasti morfologi C. nardus dan penerapannya dari perspektif tradisional Melayu, serta menentukan sebatian aktif yang hadir dalam ramuan melalui penemuan saintifik. Dua bahagian berbeza pada daun dan batang C. nardus telah diekstrak menggunakan kaedah penyulingan wap selama 2 jam. Komposisi kimia minyak citronella ditentukan melalui Kromatografi Gas - Spektrometri Jisim (GC-MS). Sejumlah 0.55% dan 0.024% minyak citronella diekstrak dari masing-masing daun dan batang, Komposit utama minyak yang diekstrak dari bahagian daun ialah citronellol (41.4%), methyleugenol (80.2%), citronellal (39.7%), D-limonene (40%) dan geraniol (36.5%). Sementara itu, pengambilan dari batang terdiri daripada α-Himachalene (45.34%), Citronellol asetat (48.2%), cis-Geraniol (57.8%) dan endo- Borneol (26.2%). Oleh itu, dapat disimpulkan bahawa pengekstrakan daun C. nardus menghasilkan kandungan minyak citronella yang lebih tinggi berbanding dengan batang dan terdiri daripada komposisi kimia utama yang berlaiman di antara kedua-dua bahagian tersebut. Selain itu, hasil kajian ini juga membuktikan bahawa kandungan fitokimia yang terdapat di dalam C. nardus mempunyai sifat anti fungal yang berkeupayaan merawat penyakit kulit dan sinus, sebagaimana aplikasi yang digunakan oleh para perawat tradisi Melayu pada abad ke 19M. Hal ini menunjukkan kebijaksanaan masyarakat Melayu lampau di
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dalam beradaptasi kamahiran hidup mereka dengan alam semulajadi sehingga mereka mampu menggembleng kesemua bahagian serai wangi untuk dijadikan sebagai ubat-ubatan.

Kata kunci: Cymbopogon nardus; Batang; Daun; Manuskrip Perubatan Melayu

1. Introduction

Flora and fauna resources in Malaysia are the contributors to the largest source of medicines in the world. The features of these plants are described in the Qur'an through surah al-An'Am verse 99, with the intent.

"And He it is Who sends down water from the sky. With it We bring forth vegetation of all kinds. Then We bring forth out of it green stalks, from which We bring forth thick clustered grain. And out of the date-palm and its sprout come forth clusters of dates hanging low and near and gardens of grapes and olives and pomegranates, each similar (in kind) yet different (in variety and taste). Look at their fruits when they begin to bear (fruit) and at its ripening. Most surely there are Signs in this for a people who believe”.

Exploration on the efficacy of herbs has been debated for a long time by traditional medical practitioners in old Malay manuscripts. The tendency to write on herbs as recorded by the Malay community shows the true philosophy and methods of practicing traditional Malay medicine. Various herbs are used in the form of mixed medicines, which are then prescribed for treating various diseases present in their daily lives, including eye disease (blurred eyes, speed points, eye tumors, conjunctivitis); ear disease (acute suppurate otitis media, inflammation in the ear); mouth ulcers, tooth problems (teeth strengthening, dental caries); respiratory illness, fever (typhoid fever); gastrointestinal tract (abdominal pain, borborigmus, dyspepsia, heartburn, medulla, constipation, indigestion, abdominal pain, dysentery), gynecological problems (uterine illness, menorrhagia, stimulative withdrawal of locomotor, postpartum disorders, hematometra, amenorrhea, leukorea) (Abd Ghani Hussain, 2015).

Various notes written by the British scholars during colonialism in Malaya before the independence phase had recorded a debate on the medical of the Malays. These include William Marsden in History of Sumatra (1811) reported list of medicinal plants and their uses; William Maxwell in Shamanism in Perak (1883); Walter Skeat, Malay Magic (1900) and Isaac Burkill, Malay Medicine in Studies from the Institute of Medical Research (1930) (Mohd Effendi, 2018). This article strives to assess the capabilities and benefits of fragrance lemongrass in the aspect of treating the disease that exists in the Malay community through the study of pharmacological-toxicological and chemical analysis.

2. Morphology of Fragrance Lemongrass and its Applications According to the views of the Malay Tradition Tradition

Fragrance lemongrass or C. nardus is a genus of Poaceae, often referred to as grassroots, fragrant and long-lived (Sukma Wardani: 2009). It is a plant of the
Graminae family, which is not similar to cooking lemongrass or Cymbopogon citratus. Cooking lemongrass is devoted to ingredients in cooking, the main supplier of ‘citral’ ingredients is used as raw material in the production of fragrances, sweets and beverages, while fragrance lemongrass is used as fragrance ingredients in the manufacture of soap, detergent and some other perfumery products (www.forestry.gov.my).

Fragrance lemongrass has reached growth up to 1 m to 1.5 m in height. The leaves have no leaf stems, simple, green, straight, layered and the average size is 60 cm x 2.5 cm. The leaves are smooth, the veins are parallel to the base and the tip of the leaves are tapered. The leaves are straight and act as stems. The rhizome is upright and strong, creeping, durable and yellowish when cut. This fragrant aroma grows widely in most countries such as Asia, especially in Malaysia, America and Africa. Fragrance lemongrass is a rugged plant and can adapt to different types of soil and climate. However, it grows well in good and fertile soil which does not live in a state of land with stagnant water and under shade. It can live lush in the open space with enough light (www.forestry.gov.my).

In Malaysia, fragrance lemongrass are traditionally used to treat rubefacient, red in the face of the skin, the root is used for treating diuretics (often occurring urine) and sudoris or diaporetics (acting as a drug to stimulate the production of sweat). The plant is also used as mosquito repellent and in making soap as well as perfumery products (http://ebuletin.mardi.gov.my).

An analysis of the old textbook in the Old Malay Manuscript illustrated by the traditional Malay medicine traditions found that fragrance lemongrass was also used frequently as a prescription in the treatment of various diseases. Among the manuscripts used as research materials are:

[1] MSS 2502 [Kitab Tibb]
[2] MSS 4231 [ Kitab Tibb & Azimat]
[3] MSS 3290 [Kitab Tibb & Ramalan]
[4] MSS 1653 [Kitab Tibb]
[5] MSS 1754 [Kitab Tibb, Azimat & Petua]
[6] MSS 33 [Kitab Tibb. Petua. Azimat]

Below are the list of diseases and the symptoms that could be treated by local wisdom prescriptions based on the Malay medical manuscripts:

- High fever medication, typhoid fever,
- Cold medication in the body
- Skin medications
- Paralyzed lung medications
- Headache medications
- Remedies
- Waist pain medications
- Skin Medication
- Genecology treatment

An analysis of the six manuscripts mentioned above found that every single parts of fragrance lemongrass were used as an ingredient in helping to treat various diseases. Reviews for every function and properties of each part of the plant are specified as below.
Part: Root of *C. nardus*

The plant has a large with short fibrous root (Figure 1). According to the views of traditional Malay medicine practitioners, the root is used as a prescription in treating the following disease (Table 1).

![Figure 1. Root of *C. nardus*](image)

Table 1. Prescription of root in treating several diseases

| Diseases          | Frequency of Usage | Part of Plant Use | References                   |
|-------------------|--------------------|-------------------|------------------------------|
| Prolong high fever | 3                  | Root              | MSS 2505,V21                 |
| Stomach ache      | 4                  | Root              | Manuskrip Melayu & Islam     |
| Typhoid           | 12                 | Root              | MS33, r72                    |
| Hernia            | 3                  | Root              | MS33, v146                   |

Part: Stem of *C. nardus*

The stems grow upright on the ground in stalks. The content of the stems are tubers, soft and hollow in yellowish or purplish white (Figure 2). However, they are rigid and easily broken. Table 2 lists several diseases which are treated by using the stem as mentioned by the traditional Malay medicine practitioners.
Table 2. Prescription of stem in treating several diseases

| Diseases        | Frequency of Usage | Part of Plant Use | References                  |
|-----------------|--------------------|-------------------|-----------------------------|
| Stomach gas     | 4                  | Stem              | Manuskrip Melayu & Islam   |
| Ulcer           | 1                  | Stem              | Manuskrip Melayu & Islam   |
| Sinustis        | 12                 | Stem              | MS33, v85                  |
| Scar removal    | 4                  | Stem              | MS33, r165                 |
| Lung            | 3                  | Stem              | MS33, v285                 |
| Geneecology     | 4                  | Stem              | MS33, v349                 |

**Part: Leaf of *C. nardus***

The leaves have rough and sharp edges with about 50-100 cm length and about 2 cm width. The leaves are green and are not stacked as shown in Figure 3. Its leafy, long, spiky leaves and form of ribbon that grow to the tip more sharp and smelly citrus when the leaves are squeezed. Based on the views of traditional Malay medicine practitioners, the leaves are used as a prescription in treating the following disease (Table 3).
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Figure 3. Leaves of *C. nardus*

Table 3. Prescription of leaf in treating several diseases

| Diseases                  | Frequency of Usage | Part of Plant Use | References                                      |
|---------------------------|--------------------|-------------------|-------------------------------------------------|
| Skin disease              | 4                  | Leaf              | MS33, v280                                      |
| Lump                      | 1                  | Leaf              | Manuskrip Melayu & Islam : r289                  |
| Bad luck elimination      | 1                  | Leaf              | MSS 3290 : Kitab Tib dan Ramalan, r44            |

Part: Flower, seed, and fruit of *C. nardus*

This type of plant rarely has flowers. Even if there is a flower, in general the flower has no crown (Figure 4) and it is seldom or even fruitless, while the seeds are also rare (Arzani and Riyanto, 1992). Based on the views of the traditional medicine practitioners, the flower is used as a prescription in treating waist pain (Table 4).

Figure 4. Flower of *C. nardus*

Table 4. Prescription of flower in treating disease
3. Materials and Methods: Scientific Analysis of *C. Nardus*

Citronella oil is a kind of essential oil that is extracted from *C. Nardus* or citronella grass and a very well known as plant-based insect and mosquito repellents (Maia et al., 2011; Koul et al., 2008; Phasomkusolsil and Soonwera, 2010; Ribeiro et al., 2016). It is a key to a new way to reduce the use of synthetic insecticide aerosol that produce harm substances, give effect to public health and pollutions to the mother nature. There are several methods to extract the oil such as steam distillation, hydro distillation and ohmic-heated hydro distillation, or new high technology methods such as supercritical fluid and microwave-assisted extraction (Abena et al., 2007; Amenaghawon et al., 2014; Cassel et al., 2009). However, new and high technology methods are more expensive, unfamiliar method of operation and require high maintenance. Thus, steam distillation or hydro distillation is the best choice to extract citronella oil from *C. nardus*. Although it is a simple method, it can induced thermal degradation hydrolysis and water solubility of some fragrance constituents (Silva et al., 2011; Hamzah et al., 2014; Kumar and Tripathi, 2011).

Basically, there are 8 constituents of citronella oil extracted by using either steam distillation, hydro distillation or ohmic-heated hydro distillation (Gahavian et al., 2012; Skaria et al., 2007; Wany et al., 2013). Chemical analysis revealed that different method of extraction resulted in different constituents of citronella oil. Hamzah and his co-researchers (2012) reported that by using ohmic-heated hydro distillation method produced 0.43 % compound of 1-ethyl-2-methylcyclopentene in the citronella oil but not from steam distillation and normal hydro distillation. In this study, steam distillation method is chosen to extract citronella oil from leaves and stems of the *C. nardus*. The percentage of oil yield and the main compounds from both parts of the plant will be determined.

**Preparation of Plant Materials**

*C. Nardus* was harvested and kept for 2 days to decrease the amount of water in the plant. The plant was dried under room temperature and not directly under sunshine to preserve the quality of the oil. Then, the dried plant was divided into two parts: leaves and stems. Each part was cut into small pieces about 3 cm to increase the surface area of the samples and exposed to the steam generated.

**Extraction of *C. Nardus***

Approximately 400 g of *C. Nardus* leaves were placed into the distillation vessel. An amount of 4 L of distilled water was poured into the round bottom flask and placed into the heating mantle, which was set on 150 °C to boil the water. The water need to be heated vigorously to create a good body of steam under pressure and to burst the cellulose of the plant to release the essential oil (Mulvaney 2010).

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| Diseases       | Frequency of Usage | Part of Plant Use | References      |
|----------------|--------------------|-------------------|-----------------|
| Waist pain     | 3                  | Flower            | MS33, v160      |
The distillation process was carried out for 2 hours from the first drop of condensed water that was collected. The liquid produced some amount of water and oil. Dichloromethane was used to enhance the separation of the oil from water. Next, the oil produced was dried using anhydrous sodium sulfate (Na₂SO₄) to remove water excess and obtain only pure citronella oil. The same method of extraction and separation was done on 600 g of the stem part of *C. Nardus*.

Each of the extracted citronella oil from both parts of the leaves and stems was analyzed using Agilent 7000A triple quadrupole GC-MS. 1µL of each sample was put into 4 different vials which was added with dichloromethane. The injector and interface were operated at 250 °C and 325 °C respectively. The oven temperature was raised from 60 °C to 325°C at a heating rate of 4 °C/min and then isothermally held for 5 minutes. The constant head pressure was 8.2317 psi. Meanwhile, the column used was a semi polar column, DB-35MS (30 m x 0.25 mm x df: 0.25µm) and the temperature was programmed to start at 50 °C to 340 °C. Mass selective detector was operated at ionization energy of 70 eV in the 35-500 amu range. The result from GC-MS was observed manually to determine the type of compound that can be found in both parts of *C. Nardus* through peak of the spectrum.

Refractive index was measured using the ATAGO-NAR-1T LIQUID at a temperature of 23.4°C and the light source used was sodium vapor lamp (589.4 nm).

4. Result and Discussion

**Extraction and Quality of citronella oil**

Table 5 shows the percentage yield of citronella oil from both parts of *C. Nardus*. From the results, it can be concluded that the amount of percentage yield of citronella oil from the leaves part is much higher compared to the stem part. During extraction process, loose packing of plant sample would improve the percentage yield of oil. However, drying the plant would not reduce the oil quantity available for extraction, but only reduce the water content in the plant. Meanwhile, the physical and chemical properties of the oil from both parts can be summarized in Table 6.

| Table 5. Percentage yield of citronella oil of *C. Nardus* leaves and stems |
|-------------------------------------------------|
| | Leaves | Stems |
| Mass of Extracted Oil (g) | 400.52 | 546.10 |
| Mass of Extracted Oil (g) | 2.212 | 0.1285 |
| Percentage of Oil Yield (%) | 0.55 | 0.024 |
Table 6. Physical and chemical properties of citronella oil of *C. Nardus* leaves and stems

| Properties          | Leaves                | Stems                |
|---------------------|-----------------------|----------------------|
| Smell               | Pungent lemon         | Fragrant lemon       |
| Colour              | Light yellow          | Colourless           |
| Refractive index    | 1.488 at 23.40°C      | 1.466 at 23.40°C     |
| Main composition (%)| Citronellol, C_{10}H_{20}O (41.4) | α-Himachalene, C_{15}H_{34} (45.34) |
|                     | Methyleugenol, C_{11}H_{14}O_{2} (80.2) | Citronellol acetate, C_{13}H_{22}O_{2} (48.2) |
|                     | Citronellal, C_{10}H_{18}O (39.7) | cis-Geraniol, C_{10}H_{18}O (57.8) |
|                     | D-limonene, C_{10}H_{16}O (40) | endo-Borneol, C_{10}H_{18}O (26.2) |
|                     | Geraniol, C_{10}H_{18}O (36.5) |                      |

**GC-MS Analysis**

Table 7-8 summarize the chemical compounds obtained from the GC-MS chromatogram from both parts of leaves and stems, respectively. It can be seen that 20 different compounds were identified from both parts based on the integration peak list produced.

Compounds present in the oil from *C. nardus* leaves are camphene, D-limonene, citronellal, isoborneol, citronellol, geraniol, geranyl acetate, methyleugenol, geraniol butyrate and hedycaryol. All compounds have Retention Time (RT) between 6.0767 - 27.8026. There are 5 compounds that show >35% of probability base matching of the spectrum with NIST library; geraniol (36.5%) (RT 18.4366), citronellal (39.7%) (RT 14.5992), D-limonene (40.0%) (RT 8.8587), citronellol (41.4%) (RT 16.8689) and methyleugenol (80.2%) (RT 25.237).

Table 7. Chemical Compounds of Citronella Oil in Leaves

| Peak | Compounds | R_T   | Height       | Area % | Probability (%) |
|------|-----------|-------|--------------|--------|-----------------|
| 1    | Camphene  | 6.0767| 253390068    | 18.41  | 26.1            |
| 2    | D-Limonene| 8.8587| 291664392    | 24.23  | 40              |
| 3    | Citronellal|14.5992| 286956238    | 27.13  | 39.7            |
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| Peak | Compounds          | RT    | Height   | Area % | Probability (%) |
|------|--------------------|-------|----------|--------|-----------------|
| 1    | Terpinolene        | 11.1681 | 222071706 | 35.79  | 20.7            |
| 2    | endo-Borneol       | 15.3896 | 234923592 | 62.08  | 26.2            |
| 3    | cis-Geraniol       | 17.2487 | 203695468 | 66.8   | 57.8            |
| 4    | L-bornyl acetate   | 19.1741 | 222848551 | 53.02  | 36.4            |
| 5    | Citronellol acetate| 20.9183 | 318598734 | 71.6   | 48.2            |
| 6    | β-Elemen           | 21.4659 | 309054250 | 59.02  | 28.3            |
| 7    | a-Himachalene      | 24.9853 | 261833418 | 100    | 45.34           |
| 8    | Citronellyl butyrate| 26.1511 | 203552923 | 33.44  | 14.6            |
| 9    | Farnesol           | 32.6294 | 264982753 | 59.16  | 35.2            |
| 10   | trans-Farnesal     | 33.8306 | 194425198 | 33.28  | 9.8             |

Table 8. Chemical Compounds of Citronella Oil in Stems

Identification of compounds from stems are found to have terpinolene, endo-borneol, cis-geraniol, L-bornyl acetate, citronellol acetate, β-Elemen, α-Himachalene, citronellyl butyrate, farnesol and trans-Farnesal. All compounds were detected between RT of 11.1681 - 33.8306. There are also 5 compounds that were detected to have >35 % of probability; farnesol (35.2 %)
Roelofs and his co researchers (2000) claimed that citral is a valuable compound which has a strong lemon odor and serves as an aroma compound and commonly used in perfumery industry. It is also widely used in the synthesis of vitamin A, flavouring and antifungal (Li et al., 2011). They observed that a compound of α-Citral was found in low percentage in C. nardus plant. The leaves contain slightly higher of citral compound compared to the stems. However, in this study, citral compound was not identified in the integration peak list of both parts as the percentage of area and probability are very low. This could be the compound that contributed to the differences of smell and appearance.

As compared to the work carried by Chong et al. (2013), the oil content of the extracted citronella oil from C. nardus is different from the present result. They reported that the composition of citronellal and geraniol were in small percentage and could not be claimed as the main compositions of oil from C. nardus leaves. They found that geraniol was the only compound with 100% area.

Based on previous research by Castro et al., (2010), elemol was one of the main compound found in the citronella oil extracted from C. nardus leaves planted in Tocantins State. However, this particular compound was not found in the extracted citronella oil from C. nardus leaves planted in Malaysia. In addition, citral compound was also not found in the citronella oil extracted in Tocantins State. This might be affected by several factors such as climate, age of plantation and efficiency of distillation method (Mulvaney 2012).

5. Conclusion

C. nardus is a kind of herb that has been widely discussed in Malay Medical Manuscript and has been consumed by Malay practitioners to cure many kinds of illness. Due to the high applications of the herb among Malay society has led us to extract the herb in identifying the bioactive compounds. The results from the study shows that C. nardus has many benefits until it is noted by previous generations that it serves to treat high fever medication, typhoid fever, cold medication in the body, skin medications, paralyzed lung medications, headache medications, waist pain medications, skin medication and gynecology treatment. The scientific studies have discovered that C. nardus leaves yield more citronella oil content (0.55%) compared to the stems part. The main compositions of oil from the leaves part are citronellol (41.4%), methyleugenol (80.2%), citronellal (39.7%), D-limonene (40%) and geraniol (36.5%). Based on the analysis conducted by the Malay manuscripts, it is found that the leaves are often used to treat skin-related illnesses. Uniquely, the phytochemical content of this lemongrass leaf can act as anti-fungal and anti-inflammatory that proves to best imply to kill bacteria (Deviliya, 2018).

On the other hand, the main compositions
of oil from the stems are α-Himachalene (45.34%), Citronellol acetate (48.2%), cis-Geraniol (57.8%) and endo-Borneol (26.2%). An analysis found by the manuscript data shows that the stems are most often used to treat sinus disease, as well as the ability to treat stomach gas, ulcer, scar removal, and genecology disease. Scientific studies have proved that the phytochemical content of the lemongrass stem capable to act as anti microbial such as acne, warts and antispasmodic that can help the respiratory and nervous systems. The citronellol compound is also able to detoxify from the body by increasing urinary frequency and sweating (Willem, 2013). This activity helps to cleanse the kidneys, liver, pancreas, digestive system, blood vessels and remove unnecessary toxins from the body through the urination process. All these facts indirectly recognize the local wisdom applied by the old Malay traditions that the methods of disease treatment are in line with the recent scientific findings. Their ability is closely related to several factors such as their habit of living close to the forest area, the wide knowledge of the plant features used as medicines and inheriting the knowledge of effective medicinal plants from their previous ancestors.

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