TEMUAN COMMUNITY OF KAMPUNG ORANG ASLI BATU 16, GOMBAK: TRADITIONAL KNOWLEDGE OF MEDICINAL PLANTS AND EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF

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FACULTY OF SCIENCE
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TEMUAN COMMUNITY OF KAMPUNG ORANG ASLI BATU 16, GOMBAK: TRADITIONAL KNOWLEDGE OF MEDICINAL PLANTS AND EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF SELECTED PLANTS

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (BIOTECHNOLOGY)

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TEMUAN COMMUNITY OF KAMPUNG ORANG ASLI BATU 16, GOMBAK: TRADITIONAL KNOWLEDGE OF MEDICINAL PLANTS AND EVALUATION OF ANTI-INFLAMMATORY POTENTIAL OF SELECTED PLANTS

ABSTRACT

The utilization of medicinal plants for the treatment of various ailments and health conditions is a common practice among the indigenous people or locally known as *Orang Asli* in Malaysia. However, traditional knowledge on medicinal plants used by *Orang Asli* has not been documented extensively. Therefore, the aim of this study was to document all the medicinal plants and their applications in treating ailments or health conditions by the Temuan tribe of Kampung Orang Asli Batu 16, Gombak, Selangor. This valuable traditional knowledge must be documented and preserved before it is lost due to modernization. Assessment of selected medicinal plants for their anti-inflammatory activity was conducted to provide evidence on the effectiveness of the medicinal plants in inflammation treatment. Data were collected from 11 respondents in the village using a semi-structured questionnaire with prior consent. Three medicinal plants such as *Molineria latifolia, Tacca integrifolia,* and *Hymenocallis speciosa* were selected for the evaluation of anti-inflammatory potential based on *in vitro* assay through inhibition of protein denaturation method. A total of 41 medicinal plant species belonging to 28 plant families were documented for their usage in the treatment of 37 ailments or health conditions. Plants of the family Zingiberaceae made up the largest number of medicinal plants (17.1%) followed by Fabaceae (9.8%) and Arecaceae (7.3%). Leaves (34.1%) are the most frequently used plant part for the preparation of herbal remedies. Although a variety of methods have been used for the preparation of herbal remedies, decoction (56.9%) is the most common method of preparation used by the Temuan tribe. In terms of mode of application, oral administration (68%) is the most common followed by being

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applied topically and bath with 14% and 10%, respectively. Bound topically (8%) is the least typical way of administration. Diabetes and fever are the most common ailments cured using medicinal plants. *Parkia speciosa* and *Ficus deltoidea* are the most commonly utilized plant species as remedies for various ailments in this study. The present findings on inhibition of protein denaturation of ethanolic and aqueous extracts of *M. latifolia* showed concentration-dependent inhibition within the range 65.63 ± 1.56% to 155.91 ± 2.15% of inhibition rate throughout the concentrations tested (100 – 500 µg/mL). Ethanolic extract of *T. integrifolia* leaves exhibited the highest (86.01 ± 4.04%) anti-inflammatory activity at a concentration of 100 µg/mL compared to their aqueous extract with only 31.88 ± 1.45% at a concentration of 300 µg/mL. Meanwhile, for extract of *H. speciosa* leaves, the aqueous extract had shown the highest suppression of protein denaturation with 87.18 ± 2.56% at a concentration of 200 µg/mL while only 42.68 ± 0.00% inhibition was observed for its ethanolic extract in the same concentration. All ethanolic extracts of these selected plants showed significant differences in inhibition activity compared to their aqueous extracts. The effect of indomethacin, a common anti-inflammatory drug, was found to be less when compared with these plant extract. Hence, the findings in this study verified the anti-inflammatory activity of the selected plants and support the claim by the Temuan that *M. latifolia, T. integrifolia,* and *H. speciosa* could alleviate their inflammation disorders.

Keywords: Temuan, traditional knowledge, medicinal plants, protein denaturation, anti-inflammatory

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KOMUNITI TEMUAN DI KAMPUNG ORANG ASLI BATU 16, GOMBAK: PENGETAHUAN TRADISIONAL BERKAITAN TUMBUHAN UBATAN DAN PENILAIAN POTENSI ANTI-RADANG BAGI TUMBUHAN TERPILIH

ABSTRAK

Penggunaan tanaman ubatan untuk rawatan pelbagai jenis penyakit dan keadaan kesihatan adalah amalan biasa di kalangan Orang Asli di Malaysia. Walau bagaimanapun, pengetahuan tradisional mengenai tanaman ubatan yang digunakan oleh Orang Asli ini tidak didokumentasikan secara meluas. Oleh itu, tujuan kajian ini adalah untuk mendokumentasikan semua tanaman ubatan dan penggunaanya dalam merawat penyakit atau keadaan kesihatan oleh suku kaum Temuan di Kampung Orang Asli Batu 16, Gombak, Selangor. Pengetahuan tradisional yang berharga ini mesti didokumentasikan dan disimpan sebelum hilang disebabkan arus pemodenan. Penilaian terhadap potensi anti-radang tanaman ubatan terpilih turut dilakukan untuk membuktikan keberkesanan tanaman ini dalam rawatan keradangan. Soal selidik separa berstruktur dilakukan untuk mengumpul data daripada 11 responden di kampung ini setelah memperolehi persetujuan mereka terlebih dahulu. Tiga tanaman ubatan seperti *Molineria latifolia*, *Tacca integrifolia*, dan *Hymenocallis speciosa* dipilih untuk penilaian potensi anti-radang berdasarkan asai *in vitro* melalui kaedah perencatan denaturasi protein. Sebanyak 41 spesies tumbuhan ubatan daripada 28 keluarga tumbuhan berjaya didokumentasikan bagi kegunaan dalam merawat 37 jenis penyakit atau keadaan kesihatan. Tumbuhan daripada keluarga Zingiberaceae merupakan tanaman ubatan paling banyak (17.1%) diikuti oleh Fabaceae (9.8%) dan Arecaceae (7.3%). Daun (34.1%) adalah bahagian tanaman yang paling kerap digunakan untuk penyediaan ubat herba. Walaupun pelbagai kaedah telah digunakan untuk penyediaan ubat herba, rebusan (56.9%) adalah kaedah penyediaan yang paling umum digunakan oleh suku Temuan. Dari segi kaedah penggunaan tanaman ubatan pula, pemberian secara oral (68%) adalah yang paling umum diikuti dengan

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penggunaan secara topikal (14%) dan mandi (10%). Terikat secara topikal (8%) adalah kaedah penggunaan yang paling sedikit. Diabetes dan demam adalah penyakit yang paling banyak dirawat menggunakan tanaman ubatan. *Parkia speciosa* dan *Ficus deltoidea* adalah spesies tumbuhan yang paling biasa digunakan sebagai tumbuhan ubatan untuk merawat pelbagai penyakit dalam kajian ini. Hasil daripada perencatan denaturasi protein, ekstrak etanol dan ekstrak air *M. latifolia* menunjukkan perencatan yang bergantung kepada kepekatan dalam lingkungan 65.63 ± 1.56% hingga 155.91 ± 2.15% kadar perencatan sepanjang kepekatan yang diuji (100 - 500 µg/mL). Ekstrak etanol bagi daun *T. integrifolia* menunjukkan aktiviti anti-radang tertinggi (86.01 ± 4.04%) pada kepekatan 100 µg/mL berbanding dengan ekstrak airnya dengan hanya 31.88 ± 1.45% pada kepekatan 300 µg/mL. Sementara itu, untuk daun *H. speciosa*, ekstrak air menunjukkan penghambatan denaturasi protein tertinggi dengan 87.18 ± 2.56% pada kepekatan 200 µg/mL sementara hanya 42.68 ± 0.00% perencatan untuk ekstrak etanol pada kepekatan yang sama. Semua ekstrak etanol tumbuhan terpilih ini menunjukkan perbezaan yang signifikan dalam aktiviti perencatan berbanding dengan ekstrak airnya. Kesan *indomethacin*, iaitu ubat anti-radang didapati kurang berkesan jika dibandingkan dengan ekstrak tumbuhan ini. Oleh itu, hasil penemuan dalam kajian ini mengesahkan aktiviti anti-radang serta menyokong penggunaan tanaman ubatan terpilih oleh suku kaum Temuan bahawa *M. latifolia*, *T. integrifolia*, dan *H. speciosa* dapat mengurangkan keradangan yang mereka alami.

Kata kunci: Temuan, pengetahuan tradisional, tumbuhan ubatan, denaturasi protein, anti-radang

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	:	Degree Celsius
p	:	Level of significance
%	:	Percent
<	:	Smaller than
±	:	Plus-minus
AE	:	Atropine equivalent
AF	:	Alkaloidal fraction
BSA	:	Bovine Serum Albumin
COX-1	:	Cyclooxygenase 1
COX-2	:	Cyclooxygenase 2
DMSO	:	Dimethyl sulfoxide
DPPH	:	2,2-Diphenyl-1-picrylhydrazyl
DW	:	Dry weight
EC_{50}	:	Half maximal effective concentration
EE	:	Ethanol extract
g	:	Gram
GAE	:	Gallic Acid Equivalent
ha	:	Hectare
HRBC	:	Human red blood cell
JAKOA	:	Jabatan Kemajuan Orang Asli
kg	:	Kilogram
km	:	Kilometre
km^2	:	Kilometre square
µg	:	Microgram
mg	:	Milligram

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mL	:	Millilitre
mm	:	Millimetre
nm	:	Nanometre
NAF	:	Non-alkaloidal fraction
NHMS	:	National Health & Morbidity Survey
NSAIDs	:	Non-steroidal Anti-inflammatory Drugs
pH	:	Potential of Hydrogen
PBS	:	Phosphate-buffered saline
S.E.M	:	Standard Error of Means
SPSS	:	Statistical Package of Social Studies

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CHAPTER 1: INTRODUCTION

1.1 Research Background

Malaysia is a tropical rainforest country that is rich with natural resources, boasting more than 20,000 species of plants. Malaysia has a great variety of medicinal plants of which almost 2,000 species have been gazetted (Lim et al., 2010). In recent years, many studies were conducted on traditional medicine, particularly medicinal plants in order to develop new drugs and medicine. Utilization of plants in traditional medicine started centuries ago and diversified across cultures and countries. In this era of modernization, some communities are still practicing traditional treatments and medicate using a variety of medicinal plants. Such medicinal plants are widely used among the *Orang Asli* in Malaysia to treat various ailments and diseases.

Most of the *Orang Asli* in Malaysia use medicinal plants as medicine because of their natural lifestyle which practice having a close relationship with the forest enabling them to have wide knowledge on the forest's resources (Ismail et al., 2015; Kardooni et al., 2014; Lin, 2005). They also practice unique lifestyles, languages, customs, and cultures. According to Lim et al. (2010), the *Orang Asli* appreciate the values of traditional herbs because they believe that these plants have potential to cure their diseases. However, the *Orang Asli* transfer this traditional knowledge on medicinal plants through verbal from one generation to another. This traditional knowledge is essential to be preserved especially among the Temuan people, who are classified under the Proto-Malay ethnic group and are largely distributed in Selangor, thus they stand to be the most vulnerable to urbanization as Selangor is the most rapidly developing state in Malaysia.

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Unfortunately, most of this valuable knowledge about the application and usage of traditional herbs has not been well documented and will be depleted due to modern medicine, demise of the older generation and lack of interest in the younger generation to learn and use the medicinal plants (Chaachouay et al., 2019; Mutie et al., 2020; Sabran et al., 2016). According to Milow et al. (2017), there are two issues that have not been sufficiently addressed in previous studies, which are the accuracy of information obtained and the extent of usage of medicinal plants by the indigenous tribes. Hence, conducting a proper survey and documentation of this traditional knowledge will be able to preserve this precious knowledge for use by future generations before it is totally forgotten, while at the same time to conserve plant biodiversity (Boadu & Asase, 2017; Ismail et al., 2015; Samuel et al., 2010).

Furthermore, evaluating the bioactive potential like anti-inflammatory effect of medicinal plants through laboratory analysis can provide evidence of the efficacy of the plants in treating the ailments or health conditions. Such finding can be used to develop new pharmaceuticals or nutraceuticals for human health. Therefore, this study focused on documenting the medicinal plants used by the Temuan community in Gombak, Selangor as well as evaluating the anti-inflammatory potential of selected medicinal plants.

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1.2 Research Objectives

The aim of this study is to document the various medicinal plants used by the Temuan people in Selangor and identify the anti-inflammatory potential of selected medicinal plants based on the specific objectives stated as follows:

(i) To document the medicinal plants used by Temuan community in Kampung Orang Asli Batu 16, Gombak, Selangor;

(ii) To document the application of medicinal plants among Temuan community in Kampung Orang Asli Batu 16, Gombak, Selangor;

(iii) To evaluate and validate the anti-inflammatory potential of selected medicinal plants based on *in vitro* method.

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CHAPTER 2: LITERATURE REVIEW

2.1 *Orang Asli*

2.1.1 Definition and distribution of *Orang Asli*

Orang Asli or indigenous people are a minority community with a population size of 178,197 in Peninsular Malaysia (Jabatan Kemajuan Orang Asli, 2016). According to Section 3 of the Aboriginal Act 1954 (Act 134), *Orang Asli* are defined as follows:

a) Anyone whose father came from the *Orang Asli* ethnic group, speak the indigenous language, practice the way of life, customs and beliefs of the *Orang Asli*, including their descendants;

b) Any person of any race adopted as a foster child by the *Orang Asli* and who has been brought up as an *Orang Asli*, habitually speaks an indigenous language, practice the way of life, customs and beliefs of the *Orang Asli*;

c) Children of any union between an *Orang Asli* female and a male from other race, speak an indigenous language and practice the *Orang Asli* way of life, customs and beliefs and remains a member of an *Orang Asli* community.

The *Orang Asli* is the earliest population in Peninsular Malaysia for their presence has been documented as early as 5,000 years ago. It is believed that most of them originated from China and Tibet, which followed the migration routes through the mainland of Southeast Asia before they stayed in the Peninsular Malaysia and the Indonesian archipelago (Masron et al., 2013).

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The *Orang Asli* are differentiated into three main ethnic groups which are Senoi, Negrito (Semang) and Proto-Malay (Figure 2.1). A census survey carried out by the Department of Orang Asli Development or *Jabatan Kemajuan Orang Asli* (JAKOA), reported the total number of *Orang Asli* in Peninsular Malaysia is 178,197. Out of this number, 97,856 individuals were from the Senoi (54.9%), 75,332 individuals from the Proto-Malay (42.3%) and 5,009 (2.8%) individuals from the Negrito (JAKOA, 2016). These three main ethnic groups are divided into 18 tribes that are distributed across Peninsular Malaysia (Figure 2.2). According to the census survey, 36.1% of the *Orang Asli* lived in the rural areas, 63.2% in marginal areas and 0.7% in urban areas.

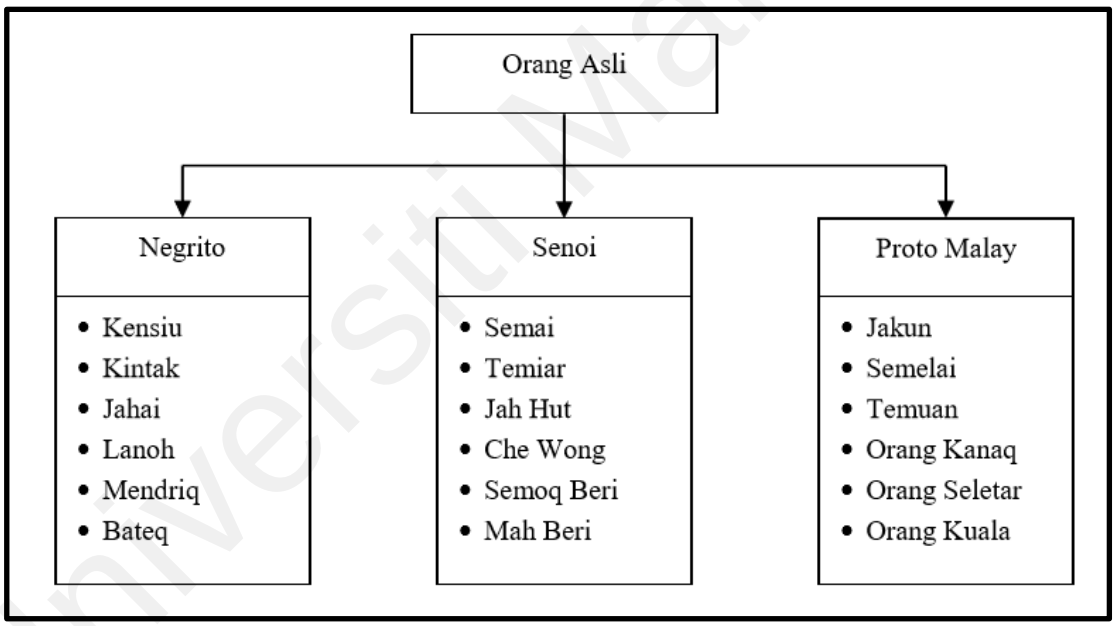

Figure 2.1: Three main ethnic groups and 18 tribes of *Orang Asli* in Peninsular Malaysia (Copyright permission from JAKOA, 2016).

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Figure 2.2: Location of 18 tribes of *Orang Asli* in Peninsular Malaysia (Copyright permission from JAKOA, 2016).

2.1.2 Temuan community

The Temuan is one of the tribes under the Proto-Malay ethnic besides Jakun, Semelai, Orang Kanaq, Orang Seletar and Orang Kuala. The features of Temuan people are identical to the Malays. They have straight hair, fairer skin and speak Malay. They also reside nearest to Malay villages. Usually, as for their source of income, they are involved in collecting and selling forest products, farming, handicrafts as well as working in government or private sectors (Said et al., 2012).

A survey made by the Department of Orang Asli Development stated that the Temuan people account for 27,590 (36.62%) individuals from the total 75,332 Proto-Malay ethnic, making them the second-highest population among the Proto-Malay ethnic group. The

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distribution of this tribe is the highest in Selangor (12,055) compared to the other states in Peninsular Malaysia such as Negeri Sembilan (7,884) and Pahang (5,220) while some of them can be found in Melaka, Johor, Perak and Kelantan (JAKOA, 2016). There were 74 *Orang Asli* villages in Selangor where four of them were located in an urban area while 70 in the suburban area. Even though they live in these areas, they still utilize the natural resources and possess the traditional knowledge of medicinal plants for treating their ailments.

2.2 Traditional Knowledge

2.2.1 Definition

Traditional knowledge can be defined as "a network of knowledge, beliefs, and traditions intended to preserve, communicate and contextualize indigenous relationships with culture and landscape over time" (Bruchac, 2014, p. 3814). Traditional knowledge is developed through experience gained over the centuries, adapted to the culture, and transmitted orally from generation to generation.

2.2.2 Importance of traditional knowledge documentation

Traditional knowledge of medicinal plants among *Orang Asli* contributes to sustainable development and play a significant role in biodiversity management and conservation of indigenous lands (Lim et al., 2010). Their skills and knowledge provide important information to the international community and could be a valuable model for biodiversity approaches until it is recognized globally. Moreover, as an indigenous community with a broad knowledge of local environments, they are most directly involved with the conservation and sustainable use of medicinal plants (Ojha et al., 2020).

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In 2016, Malaysia established the National Policy on Biological Diversity 2016-2025 which serves as a guide for biodiversity management over the next 10 years. It emphasizes the commitment of Malaysia to conserve its biological diversity, promote its sustainable utilisation and sharing benefits of the natural resources globally. It has clear goals, actions and timelines for implementation and calls for active participation by all stakeholders including *Orang Asli* in order to fulfil Malaysia's obligation under the United Nations Convention on Biological Diversity (Ministry of Natural Resources and Environment Malaysia, 2016).

According to Kardooni et al. (2014), older generations of *Orang Asli* are more knowledgeable and skilful about medicinal plants. The authors reported that 22.4% of the older *Orang Asli* generations comprising of individuals aged 50 years and above has higher level of ethnomedicinal knowledge compared to the younger generation. In addition, this can be supported by a recent study done by Mutie et al. (2020) in which, the younger generation of indigenous people have no interest to learn the knowledge of traditional medicine as they depend more on modern medicine and this has led to the inability to identify and recognize medicinal plants, and little or no knowledge of remedies at all (Sabran et al., 2016).

Thus, it is very important to record the traditional knowledge to make sure the information about medicinal plants is passed down throughout the generations. Moreover, report state that Malaysia has the world's highest rate of forest loss totalling 47, 278 km^2 area of forest land between 2000 and 2012 (Hansen et al., 2013). Hence, it is important to document this valuable traditional knowledge of medicinal plants before it becomes depleted with deforestation in Malaysia.

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2.2.3 Traditional knowledge of medicinal plants among *Orang Asli*

Various studies have been conducted on the use of medicinal plants among the *Orang Asli* in Malaysia. Samuel et al. (2010) reported 62 species of medicinal plants utilized by the Semang in Kampung Bawong, Perak while Ong et al. (2012a) documented 53 species of medicinal plants utilized among the Jah Hut tribe in Kampung Pos Penderas, Pahang. Table 2.1 shows the diversity of medicinal plants used by different tribes of *Orang Asli* in Malaysia to treat different types of ailments from simple to serious like cancer. The Temuan people also possess traditional knowledge and practically use the medicinal plants to treat their common ailments.

Most of the previous studies among the Temuan tribe only documented the plant identification as well as the application of the medicinal plants. Thus far, limited ethnomedicinal studies were conducted along with laboratory analysis to evaluate and validate the bioactivity potential of medicinal plants utilized by the Temuan tribe, particularly the anti-inflammatory potential. Ong et al. (2011a, 2011b) recorded the use of 35 and 56 species of plants, respectively, by the Temuan in Kampung Tering and Kampung Jeram Kedah, Negeri Sembilan. Total 98 species of medicinal plants were documented to be utilized by the Temuan people in Ayer Hitam Forest, Selangor (Hanum & Hamzah, 1999). In 2012, Azliza et al. reported 47 species of medicinal plants utilized by the Temuan people in Kampung Ulu Kuang, Gombak, Selangor. These studies do not adequately represent a complete picture of the utilization of medicinal plants among the Temuan tribe in Selangor. Hence, it was interesting to focus on the Temuan in Selangor for this present study because they are vulnerable to urbanization, as economic growth in Selangor is at a faster pace compared to other states in Malaysia (Department of Statistic Malaysia, 2020) and the Temuan people are highly distributed in Selangor (JAKOA, 2016).

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Therefore, more studies on the identification and evaluation of the bioactivity potential of medicinal plants in more villages of the Temuan tribe should be conducted to obtain comprehensive data and to validate the efficacy of the medicinal plants and their traditional uses.

Table 2.1: Documentation of medicinal plants utilized by *Orang Asli* in Malaysia.

Tribe	Location	Number of medicinal plant	Reference
Negrito			
Kensiu	Lubuk Ulu Legong, Kedah	39	Mohammad et al. (2012)
Semang	Kampung Bawong, Perak	62	Samuel et al. (2010)
Senoi			
Temiar	Kampung Husin, Sg Siput,Perak	18	Rachdiati & Zakariya (2018)
	Lojing Highlands, Kelantan	26	Rao et al. (2016)
	Kampung Pos Pasik, Kelantan	18	Zaki et al. (2019)
Jah Hut	Kampung Pos Penderas, Pahang	53	Ong et al. (2012a)
Semai	Kampung Batu 16, Tapah, Perak	37	Ong et al. (2012b)
Mah Meri	Kampung Orang Asli Sungai Bumbun, Pulau Carey	7	Lambin et al. (2018)
Proto-Malay			
Jakun	Kampung Peta, Johor	23	Sabran et al. (2016)
		10	Ismail et al. (2015)
Temuan	Kampung Jeram Kedah, Negeri Sembilan	56	Ong et al. (2011a)
	Kampung Tering, Negeri Sembilan	35	Ong et al. (2011b)
	Ayer Hitam Forest, Selangor	98	Hanum & Hamzah (1999)
	Kampung Ulu Kuang, Gombak, Selangor	47	Azliza et al. (2012)

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2.3 Inflammation

2.3.1 Types of inflammation

Inflammation is the normal protective reaction as a part of the host defence mechanism during forms of injury caused by noxious chemical, physical trauma or microbial agents, resulting in the swelling or edema of tissue, pain or cell damage. The importance of this mechanism is to repair and heal the damage tissue to the healthy state (Freire & Van Dyke, 2013; Oguntibeju et al., 2018).

Inflammation can be classified into two types which are acute and chronic inflammation. Acute inflammation is the immediate response and usually develops within minutes or hours after tissue injury. Acute inflammation is demonstrated by the exudation of fluid and plasma protein with the migration of leukocytes such as neutrophils. It could be characterized by five cardinal symptoms like pain, redness, heat, swelling on the affected tissue and loss of physiological functions. There are two important components in acute inflammatory process which are vascular changes and cellular events (Oguntibeju et al., 2018; Serhan et al., 2010). A few examples of acute inflammation include sprain, bone fracture, cuts, swelling and fungal infection.

Uncontrolled inflammation or prolonged acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions will cause chronic inflammation. It is manifested by the action of lymphocytes and macrophages, which lead to fibrosis and tissue necrosis (Hamidzadeh et al., 2017; Majno & Joris, 2004). Diabetes, hypertension, cancer and cardiovascular disease are some examples of diseases that also involve chronic inflammation.

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2.3.2 Anti-inflammatory activity of medicinal plants

In developing countries, most of their local or indigenous communities are still consume and depend on traditional medicines for their health care needs (Farooq et al., 2019; Ojha et al., 2020). Medicinal plants have been used by many societies, especially the indigenous people as a folk medicine to treat their ailments or diseases. They believe that medicinal plants have healing properties and the potential to be effective alternative drugs that are potentially safer than synthetic drugs (Lambin et al., 2018; Zaki et al., 2019). Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, aspirin, diclofenac, and ibuprofen are widely used in the treatment of inflammation. These drugs have great effects in inhibiting the activity of enzyme cyclooxygenase-1 (COX-1) from synthesis of prostaglandins that promote acute inflammation (Monnier et al., 2005; Oguntibeju, 2018). Nevertheless, continued use of NSAIDs can cause numerous side effects such as complications of the gastrointestinal tract and cardiovascular (Jones et al., 2008; Sostres & Lanas, 2016). Therefore, it is important to discover new medicines with anti-inflammatory properties from medicinal plants with fewer side effects and greater efficacy.

There is a wide range of natural substances and secondary metabolites in medicinal plants such as alkaloids, glycosides, amines, insecticides, steroids, flavonoids that can be used to treat diseases and have been extensively used in drugs and pharmaceutical industry. These secondary metabolites have shown to possess various biological activities including anti-inflammatory (Abu Bakar et al., 2018; Hussein & El-Anssary, 2018) and that provide a scientific base for the use of medicinal plants in traditional medicine especially among *Orang Asli*.

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Inflammation is a dynamic and complex tissue reaction provoked by cellular injury, which involves a cascade of biochemical events such as increased vascular permeability, membrane alteration and protein denaturation (Freire & Van Dyke, 2013; Serhan et al., 2010). Protein denaturation is a process in which a protein loses its secondary and tertiary structures by application of external stress or compound. Denaturation of proteins was well correlated with the occurrence of the inflammatory response that leads to multiple inflammatory disorders like arthritis (Alamgeer et al., 2017; Elisha et al., 2016). According to Opie (1962), tissue damage during life can be associated with denaturation of cellular or intercellular protein constituents. Hence, a substance's ability to prevent protein denaturation indicates the apparent potential for anti-inflammatory activity.

Previously, the effect of plants on protein denaturation has been evaluated by many researchers, for example, methanol extract of *Kalanchoe pinnata* on bovine serum albumin (Agarwal & Shanmugam, 2019), cold and hot water extract of *Ficus racemosa* bark on egg albumin (Dharmadeva et al., 2018) and methanol extract of *Barringtonia racemosa* (L) on egg albumin (Osman et al., 2016). The ability of the plant extracts to prevent thermal and hypotonic protein denaturation may be responsible for their anti-inflammatory properties. It has been proposed that the plant extract of *Berberis orthobotrys* with the presence of phenols and flavonoids might inhibit the release of the lysosomal constituents of neutrophils at the site of inflammation (Alamgeer et al., 2017). Lysosomal constituents are bactericidal enzymes and proteinases which, upon extracellular release, cause further tissue inflammation and damage (Simonaro, 2016).

Hence, inhibition of protein denaturation is representative of anti-inflammatory activity and evaluation of the anti-inflammatory potential of selected medicinal plants against the denaturation of protein was carried out in this study.

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CHAPTER 3: MATERIALS & METHODS

3.1 Chemicals and Reagents

Bovine serum albumin (BSA) and phosphate-buffered saline (PBS) were purchased from Vivantis, dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific, indomethacin was purchased from Sigma-Aldrich, and ethanol was purchased from Systerm Chemicals.

3.2 Study Location

Eight regular trips were conducted to the selected Temuan community settlement from October 2019 until December 2019. This study was conducted in a Temuan settlement named Kampung Orang Asli Batu 16, Gombak, Selangor (Figure 3.1). This study site was selected based on:

i) Recommendation from the Department of Orang Asli Development (JAKOA), Selangor among villagers who still possess traditional knowledge and use medicinal plants as therapeutics;

ii) Accessible location via transportation.

Kampung Orang Asli Batu 16 (3^{o} 19’ 27.8” N 101^{o}45’10.81” E) is one of the eight *Orang Asli* villages in the district of Gombak in Selangor. This village is surrounded by hills of green and lush plants and tranquil rivers. This village is located approximately 27 km from Kuala Lumpur city centre and is located adjacent to the Universiti Malaya Field Studies Centre, Ulu Gombak. It has a total area of about 20 ha. The average temperature in this area was 27 ºC whereby the total annual rainfall was between 2400 and 2800 mm (Malaysian Meteorological Department, 2019).

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This village is led by the village head Batin Ulang a/l Sipang. The total population of this village is about 74 individuals. The majority of the villagers here generally practice animism with a few amongst them converted to Islam. The villagers are involved in farming, collecting forest products, handicrafts, and a considerable number of them are working in government or private sectors. The infrastructure and facilities available in this village include a multifunctional hall, *surau*, tarred road, cultural stage, public toilet, electricity and water supply. Their houses are also made from concrete.

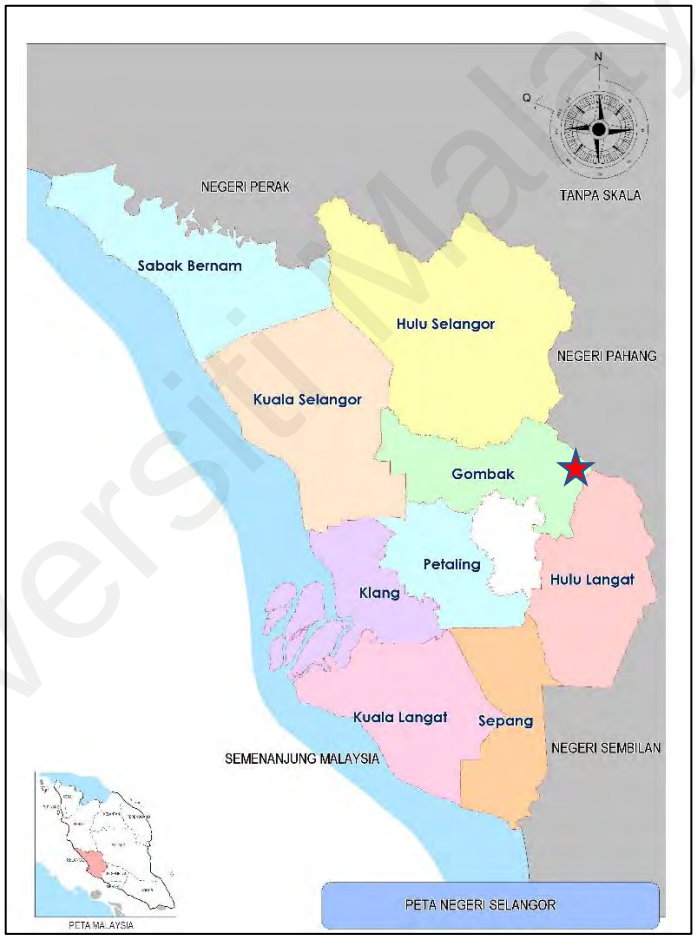

Figure 3.1: ★ Location of Kampung Orang Asli Batu 16, Gombak (Copyright permission from Selangor Town and Country Planning Department, n.d)

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3.3 Ethical Consideration

Ethical approval of this study was obtained from Universiti Malaya Research Ethics Committee (Ref No: UM.TNC2/UMREC - 662) (Appendix A) and written permission from the Department of Orang Asli Development (JAKOA) under the Ministry of Rural and Regional Development was obtained to conduct research among the *Orang Asli* community in Selangor before the commencement of the study (Ref No: JAKOA/PP.30.032Jld45(41) (Appendix B).

3.4 Respondent Selection

The respondents were selected through peer recommendation (Hu et al., 2020; Sabran et al., 2016). Respondents aged 20 years old and above, both males and females of the Temuan community were the inclusion criteria for the selection. The exclusion criteria were the respondents' consent could not be obtained, the respondents declined to participate, and respondents who are not from the Temuan tribe and not living in the study area. The participation of respondents was dependent on their self-willingness and acceptance of the terms in the consent form. Both verbal and written consent from the respondents were collected before enrolling them in the study (Appendix C). The respondents were thoroughly informed about the purpose of this study including the research objectives and methods of data collection after they gave their consent to participate in this study.

3.5 Data Collection

Data from each respondent was acquired through interviews using a semi-structured questionnaire. The questionnaire compiled demographic data of respondents and information regarding the traditional use of medicinal plants comprising common names,

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conditions treated, the parts used, methods of preparation and modes of administration (Mutie et al., 2020) (Appendix D).

3.6 Plant Identification

Plant specimens were obtained from the respondents or collected from the area. Photographs of the specimens were taken for identification and documentation. Plant identification was carried out with the help of Mr. Khairul Azmi bin Abdul Rahman from Rimba Ilmu, Universiti Malaya and by referring to various references like books and journals (Azliza et al., 2012; Hanum & Hamzah, 1999; Mohammad et al., 2012; Ong et al., 2011a; Ong et al., 2011b; Sabda, 2013; Sabran et al., 2016). Taxonomic classification and common names of plants were authenticated using online databases such as Malaysia Biodiversity Information System (MyBIS; http://www.mybis.gov.my), Global Information Hub on Integrated Medicine (GLOBinMed; https://www.globinmed.com) and The Plant List (http://www.theplantlist.org).

3.7 Evaluation of Plant Anti-inflammatory Properties

3.7.1 Plant Selection

For investigation of anti-inflammatory potential, three medicinal plants were selected namely *Molineria latifolia (lemba), Tacca integrifolia (bunga tedung)* and *Hymenocallis speciosa (kancing suasa)*. The plant specimens of *M. latifolia* and *T. integrifolia* were collected from Rimba Ilmu, Universiti Malaya whereas *H. speciosa* was collected from the Institute of Ocean and Earth Sciences, Universiti Malaya. Plants were chosen based on these criteria:

i) Plants that are utilized to treat ailments related to acute inflammation;

ii) Availability and accessibility of the plants;

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iii) No reported research has been conducted using the *in vitro* method proposed in this study on the selected plants.

The selected plant specimens were further verified by a taxonomic expert, Dr. Yong Kien Thai, from the Institute of Biological Sciences, Faculty of Science, Universiti Malaya. Voucher herbarium specimens (KLU50134: *Molineria latifolia*; KLU50135: *Tacca integrifolia*; KLU50136: *Hymenocallis speciosa*) were prepared and deposited in the Universiti Malaya Herbarium (KLU) for future reference.

3.7.2 Extraction of Plant Specimens

Plant specimens were washed and rinsed with distilled water to remove any adhering dirt and the plants were cut into small pieces. The plant specimens were dried under the sun for about 5 to 7 days to remove the moisture. Two different types of extracts were prepared using different methods of extraction namely;

a) Ethanol extract

The ethanol extracts were prepared using maceration method. Approximately 35 grams of plant specimens were soaked in 2.5L 80% ethanol for 3 days at room temperature.

b) Aqueous extract

The aqueous extracts were prepared using decoction method. Approximately 35 grams of plant specimens were boiled in 2.5L of distilled water for an hour or until the volume was reduced to half.

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After that, the ethanol and aqueous extracts were filtered separately using cotton wool and filter paper before evaporated with a rotary evaporator (Buchi Rotavapor, Switzerland) to dryness in order to yield the respective crude extracts. The crude extracts were kept in -20℃ to be used for the anti-inflammatory assay.

3.7.3 Inhibition of Protein Denaturation Assay

Inhibition of protein denaturation was done according to Bouhlali et al. (2016) with minor modification. A stock solution of 1 mg/mL was prepared freshly by dissolving the plant extracts in DMSO. The working solution was made by diluting the stock solution with distilled water to obtain the desired concentration. In this assay, BSA is used as the protein source. Denaturation of protein is induced by keeping the reaction mixture at 70℃ in a water bath for 5 minutes. The reaction mixture consists of 2.0 mL of 1% BSA prepared in PBS, pH 6.4 and 2.0 mL of varying concentrations of crude extract (100, 200, 300, 400 and 500 µg/mL). DMSO was used as a negative control while indomethacin (100, 200, 300, 400 and 500 µg/mL) was used as a positive control. The reaction mixtures were left at ambient temperature for 20 minutes and then heated at 70ºC for 5 minutes. After cooling under tap water, their absorbance was measured at 660 nm with a UV-VIS spectrophotometer (Shimadzu, Japan). The experiment was carried out in triplicates and the percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of negative control} - \text{Absorbance of test sample}}{\text{Absorbance of negative control}} \times 100$$

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3.8 Statistical Analysis

For descriptive data, frequencies of medicinal plants were determined by calculating percentages. Tables and graphs were generated in Microsoft Excel software 2016. Results for the anti-inflammatory analysis were represented as means ± standard error of means (S.E.M) and all experiments were performed in triplicates. An independent sample t-test was performed using Statistical Package of Social Sciences (SPSS) version 26.0 to evaluate the difference between the means of percentage inhibition of protein denaturation from ethanol and aqueous extract. A value of $p < 0.05$ was considered statistically significant.

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CHAPTER 4: RESULTS AND DISCUSSION

4.1 Demographic Profiles of Respondents

The indigenous community of Kampung Orang Asli Batu 16, Gombak uses diverse plants in the treatment of various ailments and health conditions. They still possess rich traditional knowledge of medicinal plants. Information about the medicinal plants was collected from the interview sessions using a semi-structured questionnaire with 11 respondents. The distribution of respondents by age, gender and educational level is shown in Table 4.1. The respondents were selected using a snowball sampling technique. The age of the respondents ranged between 36 and 68 years old. Generally, 64% of the respondents were below 50 years old. Most of the younger respondents have basic (64%) and secondary (9%) educational levels while the elder respondents age 60 to 70 years old have no formal education (27%). The male respondents are working on their own such as collecting and selling herbal and forest products, farming, and working in the government sector for their source of income. All of the 6 female respondents are housewives and some of them are making handicrafts for supporting their family income.

Table 4.1: Characteristics of the respondents

Variable	Category	Total
Gender	Female	6
	Male	5
Age	30-40	4
	41-50	3
	51-60	2
	61-70	2
Educational status	No formal education	3
	Primary	7
	Secondary	1

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The elderly respondents who are more than 50 years old possess more traditional knowledge of the medicinal plants compared to the younger respondents. They reported more numbers and applications of the medicinal plants during the interview. This same finding was also reported by Kardoni et al. (2014) among *Orang Asli* in 5 different states in Peninsular Malaysia and by Sabran et al. (2016) among Jakun people in Kampung Peta, Johor. The observed lack of knowledge of medicinal plants among younger respondents could be explained by the following reasons: lack of interest to learn about this traditional knowledge, commitment towards formal education, and assimilation to their modern lifestyles (Mutie et al., 2020; Sabran et al., 2016). Meanwhile, a study done by Hu et al. (2020) in Guangxi, China reported that some of the elder herbalists are reluctant to transfer their traditional knowledge regarding medicinal plants to the young people under the age of 30 because they feel that young people are not keen and too immature to seriously learn the valuable knowledge.

In this study, the majority of respondents have obtained low educational levels except one respondent received secondary education. Currently, educated individuals prefer and rely on modern medicine as the primary source of healthcare. Therefore, they are not interested in practising the traditional medicinal knowledge (Chaachouay et al., 2019). However, the respondent in this study who attained secondary education possess good knowledge in traditional medicine because he is the son of *Tok Batin* (head villager). The knowledge was transferred accordingly through his father and as an educated individual, it is easy for him to remember and hold the information about the medicinal plants. Nevertheless, if the knowledge is not documented properly, it will be lost along the way.

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4.2 Diversity of Medicinal Plants Species and Families

Malaysia is ranked 12th as a megadiverse country in the world due to its richness of flora and fauna (Ministry of Natural Resources and Environment, Malaysia 2016). In estimation about 20,000 species of plants with 2,000 species of medicinal plants that have been gazetted (Lim et al., 2010). It exhibits the diversity of medicinal plants in Malaysia. During this study, a total of 41 medicinal plant species used by the Temuan people of Kampung Orang Asli Batu 16, Gombak were successfully identified and documented (Table 4.2) whereas approximately 15 species could not be identified due to unavailability of the plants in the vicinity of the village. Information regarding the scientific names, local names, families, parts used, ailments treated, modes of preparation and modes of application of the plants are listed in Table 4.2 by alphabetical order according to their scientific names.

The medicinal plants recorded to be used by the villagers at the site of the study represent 28 families. Based on the list of identified plants in Figure 4.1, the highest number of medicinal plant species belong to the family Zingiberaceae (7 species; 17.1%), followed by Fabaceae (4 species; 9.8%), Arecaceae (3 species; 7.3%), Araceae and Amaryllidaceae (2 species each; 4.9%). The other 23 plant families are represented by only 1 species each. This result indicates the rich biodiversity of medicinal plants used by the Temuan people in this study. Furthermore, this observation is also supported by the studies done by other researchers demonstrating that Zingiberaceae is the most common family of herbs used as herbal medicines among Asian populations (Devi et al., 2014; Ismail et al., 2018; Mohammad et al., 2012; Ong et al., 2011c; Ong et al., 2011d; Ramli et al., 2015).

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Table 4.2: List of medicinal plants utilized by the Temuan people in Kampung Orang Asli Batu 16, Gombak.

No	Scientific name	Local name	Family name	Ailment	Part Used	Mode of preparation	Route of application
1	*Allium sativum* L.	Bawang putih	Amaryllidaceae	Diarrhoea	Rhizome	Decoction	Taken orally
				Toothache		Pounded	Applied topically
2	*Alocasia longiloba* Miq	Keladi batang hitam	Araceae	Sprain	Stem bark	Raw	Bound topically
3	*Alpinia galanga*	Lengkuas	Zingiberaceae	Skin fungal infection	Rhizome	Pounded	Applied topically
4	*Amomum aculeata*	Tepus merah	Zingiberaceae	Measles	Root	Decoction	Taken orally
				Postpartum			Taken orally
5	*Areca catechu* L.	Pinang muda	Arecaceae	Diabetes	Fruits	Decoction	Taken orally
				Hypertension			
				Dizziness		Raw	
6	*Bambusa sp.*	Buluh	Bambusa	Asthma	Juice from plant	Raw	Taken orally
7	*Calamus manan* Miq.	Rotan manau	Arecaceae	Asthma	Juice from plant	Raw	Taken orally
8	*Cinnamomum javanicum*	Pecah medang	Lauraceae	Fever	Root	Decoction	Taken orally
				Measles			Bath
				Gout	Bark	Pounded	Applied topically
9	*Curcuma longa* L.	Kunyit	Zingiberaceae	Swelling	Rhizome	Decoction	Taken orally
				Wound			

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No	Scientific name	Local name	Family name	Ailment	Part Used	Mode of preparation	Route of application
10	*Elettariopsis curtisii*	Pokok semomok	Zingiberaceae	Boils	Leaf	Pounded	Applied topically
				Wind			Bath
11	*Elettariopsis triloba*	Pokok serai gunung	Zingiberaceae	Wound	Root	Decoction	Taken orally
12	*Eurycoma longifolia*	Tongkat Ali	Simaroubaceae	Men's health	Root	Decoction	Taken orally
				Joint pain			
				Mosquitoes bite	Leaf		Bath
13	*Ficus deltoidea*	Mas cotek	Moraceae	Internal injury	Leaf	Decoction	Taken orally
				Valvular heart disease			
				Kidney stone			
				Heart disease			
				Postpartum			
14	*Flemingia strobilifera*	Daun Serengan	Fabaceae	Asthma	Leaf	Decoction	Bath
				Strengthen baby's legs			
15	*Hodgsonia macrocarpa*	Akar teruak	Cucurbitaceae	Cough	Juice from plant	Raw	Taken orally
				Fever			
				Flu			
16	*Homalomena sagittifolia*	Kemoyang	Araceae	Swelling	Rhizome	Decoction	Taken orally
				Postpartum	Leaf	Raw	Bound topically

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No	Scientific name	Local name	Family name	Ailment	Part Used	Mode of preparation	Route of application
17	*Hymenocallis speciosa*	Kancing suasa	Amaryllidaceae	Swelling	Leaf	Pounded	Bound topically
				Sprain			
18	*Kalanchoe pinnata* *Syn: Bryophyllum pinnatum*	Daun sedingin	Crassulaceae	Fever	Leaf	Decoction	Taken orally
19	*Labisia pumila* (Blume) Merr	Kacip Fatimah	Myrsinaceae	Postpartum	Root	Decoction	Taken orally
20	*Lansium domesticum* Corr	Duku	Meliaceae	Stomach ache	Fruit peel	Decoction	Taken orally
21	*Mikania micrantha* Kunth	Ulan	Asteraceae	Wound	Leaf	Pounded	Applied topically
22	*Molineria latifolia* (Dryand. ex. W. T. Aiton) Herb. ex Kurz	Lemba'	Hypoxidaceae	Swelling	Fruits	Decoction	Taken orally
				Fever			
				Haemoptysis			
				Wound	Leaf	Pounded	Applied topically
				Centipede bites		Raw	Bound topically
23	*Muntingia calabura*	Pokok ceri	Muntingiaceae	Diabetes	Stem bark	Decoction	Taken orally
				Stroke	Leaf		
24	*Musa paradisiaca*	Jantung pisang	Musaceae	Increase breast milk	Fruits	Cooked	Taken orally
25	*Nephelium mutabile*	Pulasan	Sapindaceae	Stomach ache	Fruit peel	Decoction	Taken orally

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No	Scientific name	Local name	Family name	Ailment	Part Used	Mode of preparation	Route of application
26	*Nicotiana tabacum* L.	Tembakau	Solanaceae	Diarrhoea	Leaf	Decoction	Taken orally
27	*Orthosiphon aristatus*	Misai kucing	Lamiaceae	Hypertension	Leaf & Stem	Decoction	Taken orally
				Diabetes			
				Sprain			
28	*Parkia speciosa* Hassk	Petai	Fabaceae	Diabetes	Root	Decoction	Taken orally
				Hypertension			
				Dizziness			
				Diarrhoea			
				Gout			
29	*Phyllanthus amarus*	Pokok dukung anak	Euphorbiaceae	Jaundice	Leaf	Decoction	Bath
30	*Pinanga kuhlii*	Pinang hutan	Arecaceae	Cough	Fruits	Raw	Taken orally
				Flu			
				Fever	Leaf & Root	Decoction	Bath
31	*Poikilospermum suaveolens* (Blume) Merr	Akar setawan	Cecropiaceae	Fever	Juice from plant	Raw	Taken orally
				Measles			
				Cough	Root	Decoction	
32	*Polyalthia bullata*	Tongkat Ali hitam	Annonaceae	Cough	Root	Decoction	Taken orally

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No	Scientific name	Local name	Family name	Ailment	Part Used	Mode of preparation	Route of application
33	*Psidium guajava* L.	Jambu batu	Myrtaceae	Diabetes	Leaf	Decoction	Taken orally
				Hypertension			
				Dizziness			
				Diarrhoea		Raw	
34	*Rafflesia hasseltii*	Bunga pama	Rafflesiaceae	Haemorrhoid	Petal	Decoction	Taken orally
35	*Rourea concolor*	Akar semelit	Fabaceae	Diabetes	Root	Decoction	Taken orally
				Kidney disease			
				Waist pain			
36	*Saraca declinata* Miq / *S. thaipingensis*	Pokok kapih	Fabaceae	Diabetes	Leaf	Raw	Taken orally
37	*Smilax myosotiflora* ADC	Ubi jaga	Liliaceae	Muscle pain	Rhizome	Decoction	Taken orally
				Difficulty to walk (baby)			
38	*Tacca integrifolia*	Bunga tedung/ Bunga belimbing	Taccaceae	Waist pain	Rhizome	Decoction	Taken orally
				Swelling	Leaf		Taken orally & bath
39	*Uncaria callophylla* Blume	Akar kelait/ akar kait2	Rubiaceae	Cough	Root	Decoction	Taken orally
40	*Zingiber montanum*	Bonglai	Zingiberaceae	Gout	Rhizome	Pounded	Applied topically
				Itch			
41	*Zingiber officinale*	Halia	Zingiberaceae	Postpartum Flatulence	Rhizome	Pounded	Taken orally

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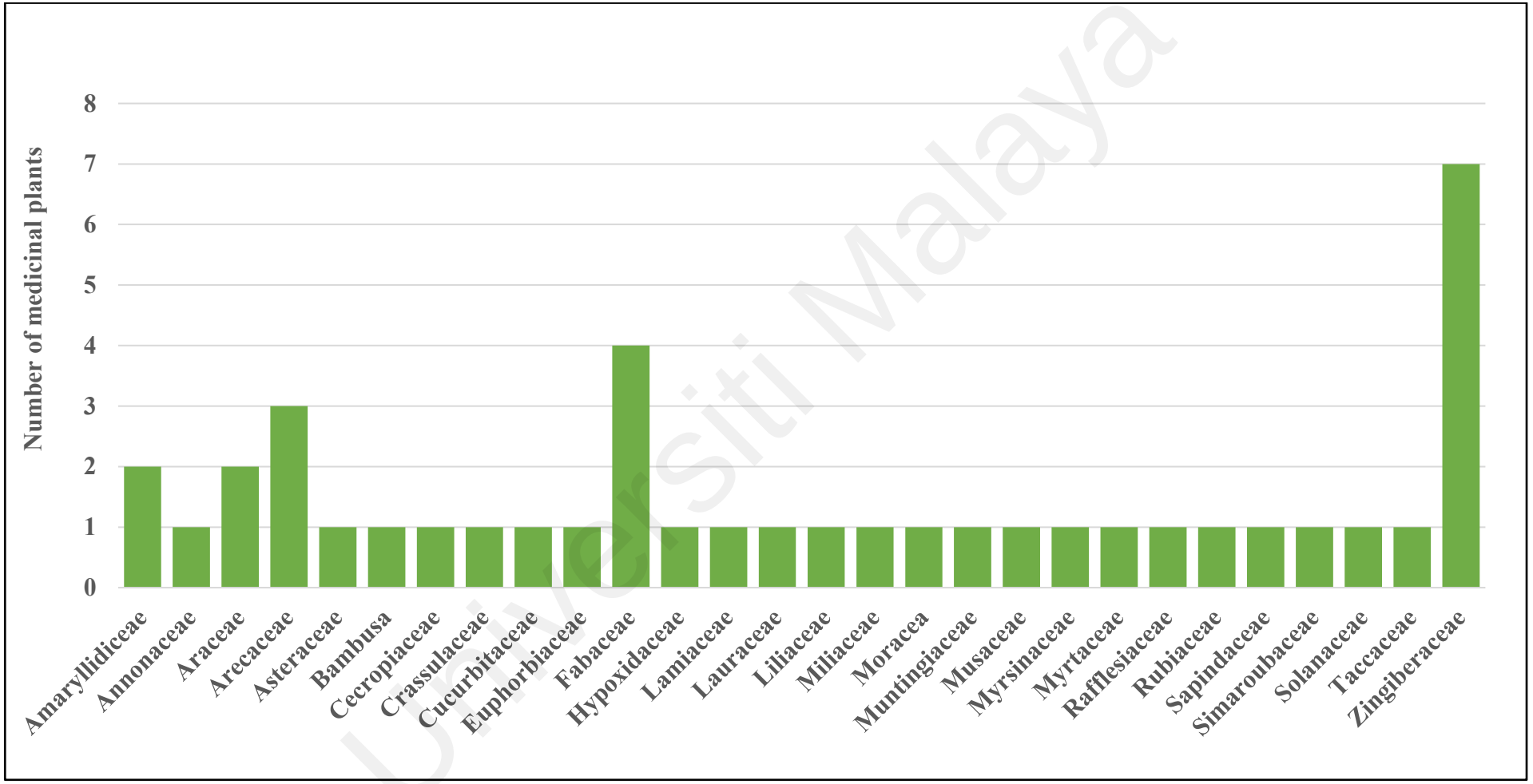

Figure 4.1: Families of medicinal plants in Kampung Orang Asli Batu 16, Gombak.

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Most of the species from the Zingiberaceae family are economically valuable and important natural resources for their spices, natural dyes, perfumes and herbal medicines (Devi et al., 2014; Mans et al., 2019). These aromatic herbs grow in the humid areas of the tropics and subtropics, including some seasonally dry regions. It is distributed across tropical Africa, Asia and the Americas with the greatest diversity in Southeast Asia (Jatoi et al., 2007). This family includes 52 genera and approximately 1,600 known species of perennial flowering plants that are variable in height and size (Christenhusz & Byng, 2016). In Peninsular Malaysia, there are approximately 160 species of Zingiberaceae belonging to 18 genera (Larsen et al., 1999).

Generally, the species in this family have sympodial branched rhizomes and composed of different segments. The young rhizomes and axillary buds are protected by scale leaves. Leafy shoots may reach up to 5 metres in height are generally unbranched and true aerial stem is present in some genera and absent in others. True stem is very short as in *Kaempferia* or pseudostem with clasping leaf sheaths as in *Curcuma*. The leaves are distichous and they exhibit morphological variation in structure, shape, size, texture and venation (Tomlinson, 1956).

In this present study, *Alpinia galanga (lengkuas), Amomum aculeata (tepus merah), Curcuma longa (kunyit), Elettariopsis curtisii (semomok), Zingiber montanum (bonglai) and Zingiber officinale* (*halia*) have been cultivated by the Temuan people surrounding their home gardens for their spices, condiments and herbal medicines. These plants are reported as the most important plants with medicinal uses in Malaysia under Zingiberaceae family (Ibrahim et al., 2000). The traditional uses of these species may be attributed to the presence of various phytochemicals in the rhizomes and leaves with meaningful pharmacological activities.

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For example, a previous study reported the essential oils in both leaves and rhizomes of *E. curtisii* (Figure 4.2) have been analysed as aldehydes with (E)-2-octenal, (E)-2-decenal and (E)-2-decenoic acid as the most abundant component (Ibrahim et al., 2009). According to Chairgulprasert et al. (2008) the essential oils from leaves and rhizomes of *E. curtisii* displayed the highest inhibitory activity against 5 strains of bacteria, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Sarcina sp*. and *Pseudomonas aeruginosa* while the crude dichloromethane extract of the leaves exhibited the highest scavenging effect on the DPPH radical with an EC_{50} of 0.28±0.01 mg/mL. Therefore, this finding supports the traditional usage of this plant, particularly in the treatment of bacterial infections among the Temuan people in this present study because they believe that the leaves of *E. curtisii* can treat boils which are caused by bacteria infection by pounding and applying it topically. They also consume the leaves as a food flavouring in their dishes preparation because it will release a delicious aroma when cooked.

Figure 4.2: *Elettariopsis curtisii* (Photo taken at Kampung Orang Asli Batu 16, Gombak).

Curcuma longa better known as turmeric or *kunyit* in Malaysia is a popular plant under Zingiberaceae with multiple uses. It has been utilized for generations in food preparations especially in Asian cuisine and as traditional remedies to treat various ailments and health conditions across the world (Hewlings & Kalman, 2017; Jamal et al., 2011). The rhizome of *C. longa* contains the most active and essential constituent which is curcumin, a polyphenol (Singletary, 2010). This active substance was reported to possess potential health benefits as a healing agent for various diseases. A randomized, double-blind, placebo-controlled study done by Small et al. (2018) reported that daily oral intake of bioavailable form of curcumin (Theracurmin® containing 90 mg of curcumin) improved memory and attention in adults without dementia. There is also supporting evidence that curcumin administration is safe, well-tolerated, and showed highly significance in the reduction of depressive symptoms in patients with major depression (Al-Karawi et al., 2016). A systematic review done by Garg et al. (2012) suggest that curcumin might be an effective and safe therapy for maintenance of remission in ulcerative colitis when administered as an additional therapy with mesalamine or sulfasalazine. To attain the efficacy of this curcumin, it must be combined with other active components from other plants to increase the bioavailability and provide multiple health benefits (Hewlings & Kalman, 2017).

Similarly, the Temuan people in this study decocted the *C. longa* rhizome with other plants which are leaves of *M. latifolia* (*lemba*) to treat swelling and wound while the rhizome of *C. longa* is pounded and applied on the forehead to cure fever. Previously, an *in vivo* study of nasal mucosa trauma by Emiroglu et al. (2017) reported that treatment with 10 mg/mL curcumin reduced inflammatory response and significantly accelerated wound healing. Besides that, numerous extensive studies either *in vitro* or *in vivo* together with clinical trials have discovered the important biological activities related to its anti-

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inflammatory, antioxidant, antiviral and cancer-preventive properties that have been discussed in this review (Rathore et al., 2020). These findings support the traditional usage of *C. longa*.

4.3 Ailments and Health Conditions Treated

Overall, a total of 37 ailments and health conditions was treated by the Temuan people solely by using plant species obtained from their surroundings. The Temuan people in this study not only utilize medicinal plants for the treatment of simple ailments and health conditions like stomach ache, toothache, postpartum, inflammation including swelling, sprain, wound and infection but also to treat chronic diseases such as stroke, diabetes and hypertension. Diabetes and fever are the most common treated conditions among all the reported ailments in this study. Seven species of medicinal plants are used to treat diabetes followed by 6 species for fever treatment.

Diabetes is a metabolic disorder characterized by high blood glucose levels or hyperglycaemia. According to the National Health and Morbidity Survey (NHMS) in 2019 conducted by the Ministry of Health Malaysia, the prevalence of diabetes among Malaysians was 18.3% (Institute of Public Health, 2020). A previous finding reported the prevalence of diabetes among *Orang Asli* in Malaysia are 2.1% to 9.1% (Phipps et al., 2015). Even though they have access to modern medical treatments for their diabetic problem, some of the elder generation in this study still rely on medicinal plants. The preparation of herbal remedies for diabetes treatment in this village is mostly in the form of decoction and administered orally. *Muntingia calabura* (*pokok ceri*), *Rourea concolar* (*akar semelit*), *Saraca thaipingensis (pokok kapih*), *Areca catechu* (*pinang muda*), *Psidium guajava* (*jambu batu*) and *Parkia speciosa* (*petai*) are the medicinal plants reported by the villagers for diabetes treatment.

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Parkia speciosa Hassk or stink bean, commonly known as *petai* in Malaysia is belong to the Fabaceae family. It is one of the most utilized plant species by the Temuan people in this village to treat the highest number of ailments and health conditions such as hypertension, dizziness, diarrhoea, gout and diabetes as well. According to one of the respondents, the root of *P. speciosa* must be decocted with the fruit of *A. catechu* and the leaf of *P. guajava* to maximize the effectiveness to treat diabetes. However, other Temuan tribes in Selangor and Negeri Sembilan (Azliza et al., 2012; Ong et al., 2011b) as well as the Semai tribe in Perak (Ong et al., 2012b) utilize the decoction of the root of *P. speciosa* alone to treat this ailment. Research done by Jamaluddin & Mohamed (1995) reported antidiabetic activity in the chloroform extract of *P. speciosa*. Administration of the extract in alloxan-induced diabetic rats produces a significant reduction in blood glucose level. This finding supports the traditional use of *P. speciosa* in diabetes treatment. Other scientific findings had also revealed the antioxidant, antitumor, and antimicrobial activity of the *P. speciosa* in the reviewed article by Kamisah et al. (2013).

Fever is generally defined as a rise of human body temperature above normal range that is 37°C. The Temuan people in this study utilize six species of medicinal plants namely *Cinnamomum javanicum* (*pecah medang*), *Hodgsonia macrocarpa* (*akar teruak*), *Molineria latifolia* (*lemba*), *Pinanga kuhlii* (*pinang hutan*), *Poikilospermum suaveolens* (*akar setawan*) and *Kalanchoe pinnata (daun sedingin)* in treating fever including. *K. pinnata* is a succulent plant that grows up to 1.5 meters tall and commonly known as cathedral bells or *daun setawar* among Malays. The leaves are thick, glabrous and elliptical in shape (Figure 4.3). They decoct the leaves with a small amount of turmeric and uncooked rice (*beras*) before drinking it. They believe that this can reduce the temperature of their body and make them feel better. The Malays community in Perak

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and Terengganu also utilize the leaves of *K. pinnata* by pounding and applying it onto the forehead to treat their headaches (Ong et al., 2011c; Ramli et al., 2015).

Figure 4.3: *Kalanchoe pinnata* (Photo taken at Kampung Orang Asli Batu 16, Gombak).

4.4 Parts Used

Every parts of the medicinal plants possesses its advantages in order to treat various and different ailments. In the present study, direct communications with the Temuan people revealed that different parts of the plants are used for the preparation of herbal medicine. Among all the plant parts, leaves are commonly used, which is represented by 34.1% of all listed plants (Table 4.2). The second most common part being used for medicinal practices is the root, making up 20.5% followed by rhizome (18.2%), fruits (9.1%), juice from root/stem (9.1%), stem bark (4.5%) and fruit peel (4.5%) (Figure 4.4).

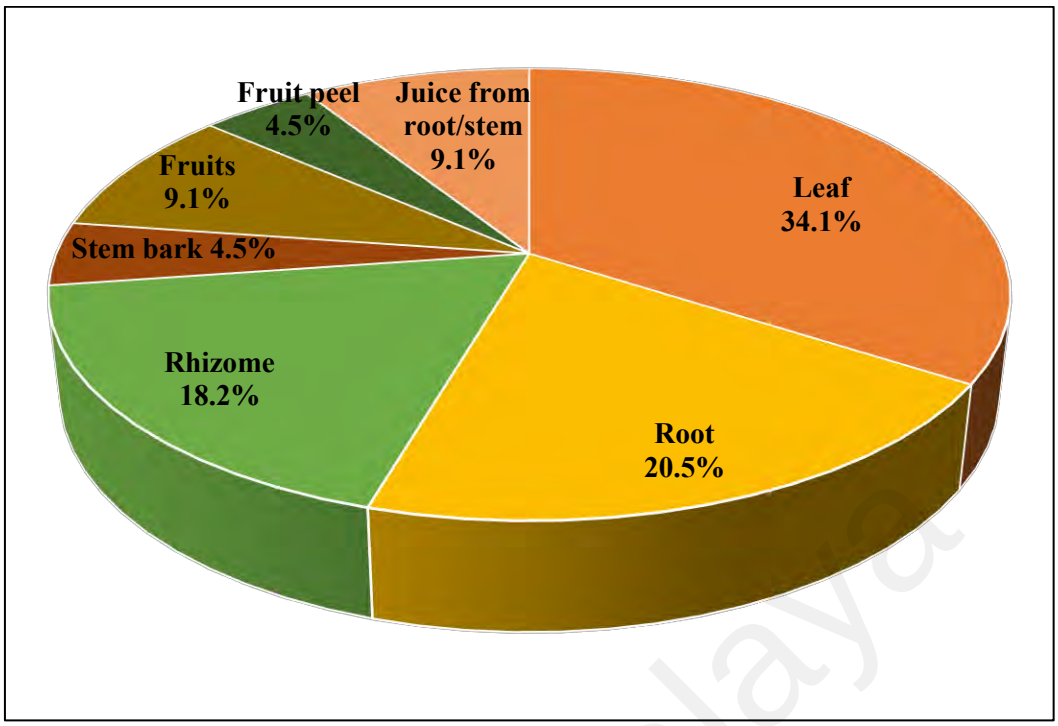

Figure 4.4: Percentage of plant parts for 41 medicinal plants used in Kampung Orang Asli Batu 16, Gombak.

Ethnomedicinal studies on different tribes of *Orang Asli* which are the Semai tribe in Perak and the Temiar tribe in Kelantan also indicated leaves as the frequently utilized part used in herbal medicine (Ong et al., 2012b; Rachdiati & Zakariya, 2018; Rao et al., 2016). Similarly, the utilization of the leaves for various health problems is in agreement with many ethnomedicinal studies in Peninsular Malaysia and abroad with 55.5% in Terengganu (Abdulrahman et al., 2018), 52.7% in Johor (Ismail et al., 2018), 39.13% in India (Rana et al., 2014), 29% in Pakistan (Farooq et al., 2019) and 26% in Egypt (Mahmoud & Gairola, 2013).

The preference of using the leaves compared to other plant parts might be due to the benefit of medicinal properties and also the easy accessibility and abundant availability of the leaves (Chaachouay et al., 2019; Migueis et al., 2019). In addition, the leaves are directly involved in photosynthesis as well as the primary reservoir of a high number of secondary metabolites. A phytochemical study of selected medicinal plants in Central Sulawesi, Indonesia reported that the leaves were rich with flavonoids, tannin, saponins,

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alkaloids and terpenoids that contribute significantly to the medicinal value of the plants (Ramadanil et al., 2019).

For instance, the leaves of few plants reported in this study such as *Ficus deltoidea* (*mas cotek*), *Orthosiphon aristatus* (*misai kucing*), and *Phyllantus amarus* (*dukung anak*) have been used in traditional medicine and commercialized herbal products in Malaysia (Lim et al., 2010). Besides, *F. deltoidea* is one of the most utilized medicinal plants by the Temuan in this study for treating their ailments such as heart disease, kidney stone, internal injury and valvular heart disease. Leaves of *F. deltoidea* are traditionally used by the Temuan in this study for post-partum treatment and maintaining women's health. Previous studies on the Malays in Peninsular Malaysia also reported the traditional usage of this plant in treating hypertension and diabetes (Abdulrahman et al., 2018; Ismail et al., 2018; Ramli et al., 2015). On top of that, the dried leaves of *F. deltoidea* are often commercialized as herbal tea, containing a large quantity of magnesium, manganese and potassium that can serve as a good source of minerals for human consumption (Mohammad et al., 2012).

Besides, the root is also the one of the most favoured plant parts in this study, possibly due to the role of the roots as food and nutrient storage, so they also have a high content of bioactive constituents. The roots of *Labisia pumila* and *Eurycoma longifolia* are examples of medicinal plant species that are rich in medicinal value and economic importance to the nation. *L. pumila*, locally known as *kacip Fatimah* (Figure 4.5) and *E. longifolia*, known as *tongkat Ali* among locals in Malaysia, have been widely used among Malaysians for decades for numerous ailments and are commercially produced as food supplements. Traditionally, the root extract of *L. pumila* is consumed by women of the Temuan in this study for post-partum treatment whereas the root extract of. *E. longifolia*

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is consumed by men as an aphrodisiac and joint pain. Various researchers also reported similar functions of these plants used by Malays and other tribes of *Orang Asli* in Malaysia (Baling et al., 2017; Ismail et al., 2018; Mohammad et al., 2012). The root extract of *L. pumila* also has been reported to have anti-inflammatory, anti-fungal and cytotoxicity activity (Karimi et al., 2013; Sanusi et al., 2013). A clinical study on *L. pumila* has also reported that this plant extract has the potential to treat postmenopausal symptoms (Abdul Kadir et al., 2012).

Figure 4.5: *Labisia pumila* (Blume) Merr (Photo taken at Kampung Orang Asli Batu 16, Gombak).

Furthermore, the Temuan people in this study believe that different parts of the same plant may have different medicinal efficacy. The root of *Cinnamomum javanicum* (*pecah medang*) for example is decocted and taken orally for fever and measles treatment while paste made from the bark is applied to treat gout. The respondents also mentioned that different parts of different plants can have the same medicinal function. For instance, rhizome of *Homalomena sagittifolia* (*kemoyang*), fruits of *M. latifolia* (*lemba*) and leaves of *T. integrifolia* (*bunga tedung*) can be utilized to treat swelling.

However, the frequent utilization and over-harvesting of the plant parts that have high commercial and medicinal value in the community, may pose a serious threat to biodiversity conservation. Collection of the leaves prevents the conversion of vegetative to reproductive development which in turn inhibits the plant's growth. On the other hand, uprooting medicinal plants cause total destruction of the plants (Tugume et al., 2016). Therefore, attention to conservation should be prioritized to ensure the sustainable utilization of these plants by domestication and cultivation on a large scale.

4.5 Preparation and Administration of Medicinal Plants

Various methods can be used in the preparation of herbal medicines to get the benefits in treating various ailments and health conditions. In terms of preparation of herbal remedies in this Temuan community (Figure 4.6), decoction is the most frequent method used which applies to 29 species (56.9 %) of the plants documented. Other common methods of preparation are eaten and applied raw (11 species; 21.6 %), pounded (10 species; 19.6 %), and cooked as food for only one species (2.0 %).

Decoction is a method to extract the bioactive molecules by boiling the plant material. The frequent use of the decoction can be explained by the fact that the effectiveness of herbal remedies increases in decoction due to maximum extraction of bioactive compounds such as terpenoids, tannins, anthocyanins, and saponins which is accelerated by heating (Azmir et al., 2013).

Meanwhile, mode of administration also varies depending on ailments and health conditions. The most typical mode of administration (Figure 4.7) in this study is taken orally which is applicable to a total of 34 species (68%) followed by applied topically and bathing with 14% and 10% respectively. The least typical way of administration is bound topically which is applicable to only 5 species (8%).

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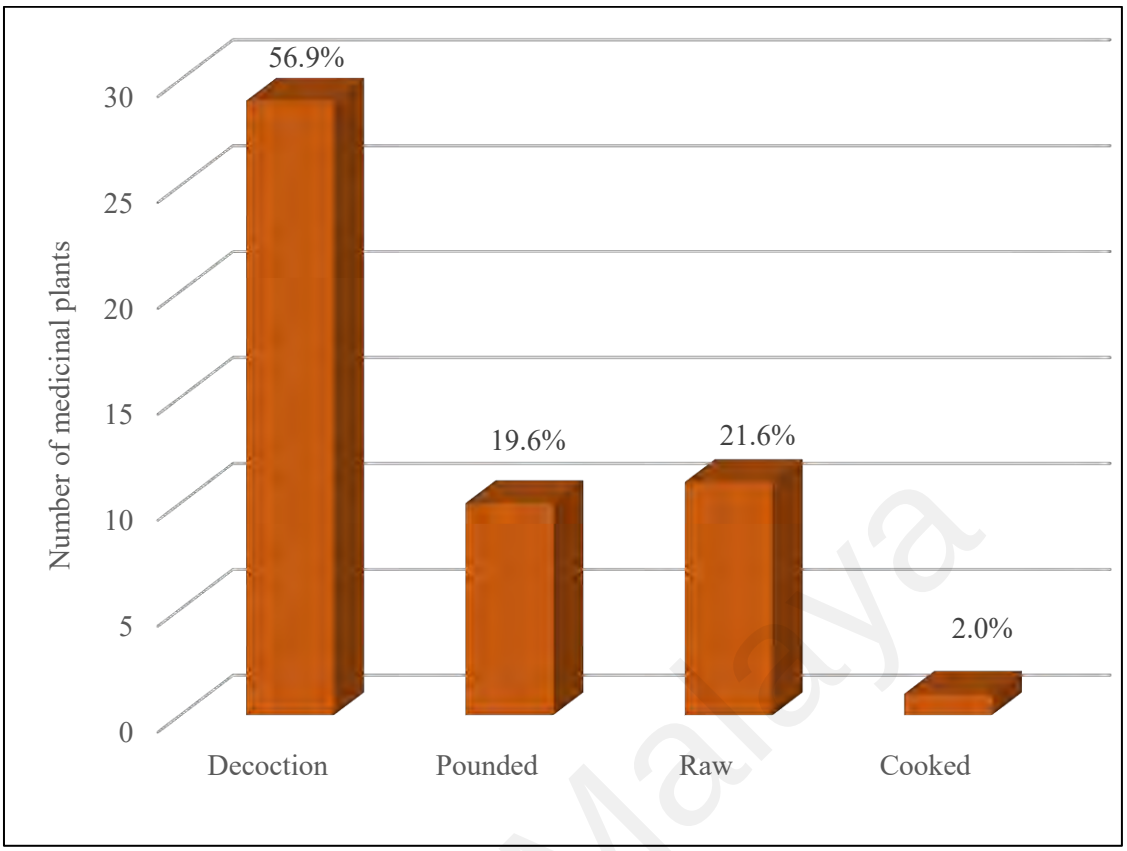

Figure 4.6: List of the mode of preparation

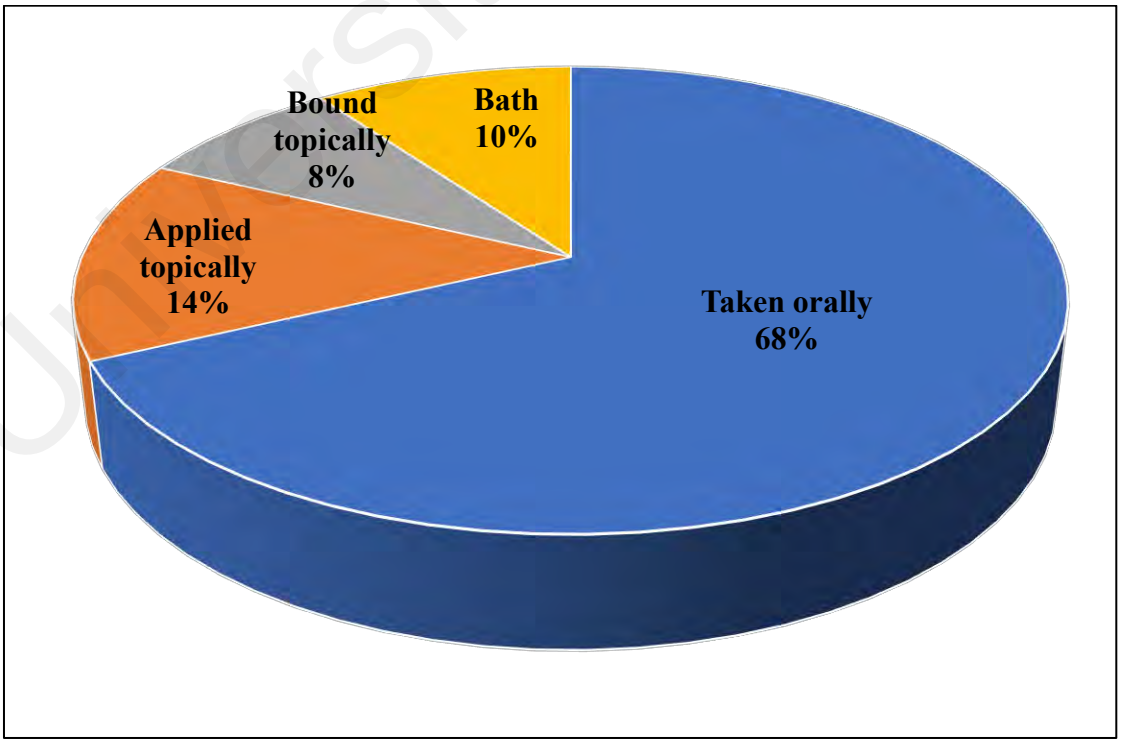

Figure 4.7: Percentage of mode of administration for 41 medicinal plants used in Kampung Orang Asli Batu 16, Gombak.

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The fact that the medicinal plants are orally consumed is the most common is not surprising as this has been previously reported in numerous ethnomedicinal studies (Abdulrahman et al., 2018; Boadu & Asase 2017; Chaachouay et al., 2019). Oral administration of herbal preparation is preferred over the other administration routes because of the many advantages it presents. These benefits include safety, ease of ingestion and pain avoidance (Sastry et al., 2000). Besides, oral intake of herbal preparation is usually effective for the treatment of internal diseases, while for external diseases such as cuts, boils, sprain, and skin fungal infection are treated by topical application of the herbal remedies.

Root decoction of *Polyalthia bullata* (*tongkat Ali hitam)* is an example of a plant that is consumed orally by the Temuan in this study to alleviate cough. Meanwhile, the Malays of Kangkar, Pulai (Alsarhan et al., 2012) and the Jah Hut tribe of Kampung Penderas, Pahang (Ong et al., 2012a) decoct the roots of *P. bullata* and consume as an aphrodisiac. The Temuan in this study also decoct the leaves and other parts of the plant for their herbal preparation. According to *Tok Batin*, to get the efficacy of the medicinal plants, the plant parts must be boiled in water for a certain period of time until the volume of the water is reduced to minimum amount. To make the taste more pleasant to drink and minimize discomfort, a little amount of sugar or honey can be added.

Besides consuming the decoction of the medicinal plants orally, they also can be used topically as herbal baths such as decoction of *Phyllantus amarus* (*dukung anak*) leaves for jaundice treatment, decoction of *Pinanga kuhlii* (*pinang hutan*) leaves and roots for fever treatment, and decoction of *Cinnamomum javanicum* (*pecah medang*) root for measles treatment.

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Pounding the parts of medicinal plants and applying them topically onto the pain or affected area is also the mode of preparation and administration of their herbal remedies among the Temuan people in this study. *Allium sativum*, also known as garlic *(bawang putih)* is pounded with a pinch of salt before applying to treat toothache. *A. galanga (lengkuas)* is utilized by pounding the rhizome and applying it to their skin afflicted with tinea versicolor or pityriasis versicolor, which is a common fungal infection of the skin among teens and young adults (Dourmishev, 2020). This condition is known as *panau* locally. It is not contagious or painful but skin discoloration can lead to emotional distress and self-consciousness (Bamford et al., 2018). The Malay community believes that this plant not only can treat skin infections but also flatulence and vomiting (Ong et al., 2011c; Ong et al., 2011d) due to the antifungal and digestive stimulant properties of the rhizomes (Chouni & Paul, 2018). Furthermore, according to one of the respondents, they used the raw plant to treat their ailment by bounding it topically to the affected area. The stem bark of *Alocasia longiloba* (*keladi batang hitam*) is used to bind the legs or hands before they wrap it with cloth for sprain treatment (Figure 4.8).

Figure 4.8: *Alocasia longiloba* (Photo taken at Kampung Orang Asli Batu 16, Gombak).

Additionally, even though only one species of plant was recorded as cooked for the mode of preparation for herbal remedies, it is frequently utilized among women in this village. The women respondents in this study believe that consumption of cooked *jantung pisang* or inflorescence of *Musa paradisiaca* will increase milk production for women after birth. According to Mahmood et al. (2012), flower extract of *M. paradisiaca* has been proven to be effective for breast milk production on lactating rats by 25%. A previous study on phytochemical constituents in the *M. paradisiaca* flower showed that it contains galactagogue compounds such as alkaloids, saponins, glycosides, tannins, flavonoids, and steroids that have the potential to stimulate the hormones oxytocin and prolactin, which boosts milk production (Othman et al., 2014). Another study from Indonesia by Laeli et al. (2018) also reported a similar finding whereby consumption of banana flower contributed to a significant increase in prolactin hormone levels and milk production in postpartum mothers.

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4.6 Evaluation of Plant Anti-inflammatory Properties

4.6.1 Selection of medicinal plants

Medicinal plants represent an important part of disease remedies for the *Orang Asli* in Malaysia. The Temuan in this study believe that medicinal plants have healing properties and potential to be effective alternatives drugs. Among all of these documented medicinal plants, there are several plants used by the Temuan tribe in this village to treat acute inflammation such as wound, cuts, swelling, sprains, bone fracture, and skin infection. Redness, heat, pain, and loss of function were the signs of the acute inflammation which occurs immediately as a defense mechanism of the body to fight against any injuries, infections, and allergic reactions. This mechanism is important to repair and heal the damage tissue to the healthy state (Freire & Van Dyke, 2013).

Some of the medicinal plants used for acute inflammation treatment among different tribes of *Orang Asli* such as *C. longa (*Al-Karawi et al., 2016; Garg et al., 2012; Small et al., 2018; Singletary, 2010), *A. galanga* (Jatoi et al., 2007; Ramadanil et al., 2019) and *H. sagittifolia* (Eldeen et al., 2016; Wong et al., 2012) have been thoroughly investigated on their anti-inflammatory activity. However, from the data we collected, three medicinal plant species shortlisted for the evaluation of anti-inflammatory assay including *M. latifolia, T. integrifolia* and *H. speciosa* have not been well studied. The following sections describes these herbs.

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4.6.1.1 *Molineria latifolia*

Figure 4.9: ***Molineria latifolia*** **(Photo taken at Kampung Orang Asli Batu 16, Gombak).**

Molineria latifolia or its synonym *Curculigo latifolia* is categorized under the family of Hypoxidaceae. It can be found natively in tropical areas such as Malaysia and other Southeast Asian countries such as Thailand, Indonesia, Myanmar and Philippines. This species grows in slightly shady or sunny patches of lowland forest or hills. This plant is commonly known as weevil lily or *lemba* in Malaysia (Figure 4.9). The Malays have widely utilized the leaves of *M. latifolia* for swelling and wound healing treatment (Sabda, 2013), while the Temuan in this study use the leaves of *M. latifolia* to treat wounds and the fruits of *M. latifolia* for treatment of swelling.

Besides, the Temuan in this study also utilize the fruits to treat fever and haemoptysis by decoction of the fruits with turmeric. They use the leaves to aid in wound healing by pounding and applying it to the affected skin area. They also love to consume the fruits because of the sweet taste due to the presence of a protein known as neoculin in *M.*

latifolia fruits. Neoculin is a sweet protein with a taste-modifying activity that turns sourness into sweetness (Okubo et al., 2008). Previous studies reported that *M. latifolia* has numerous therapeutic activities such as antioxidant (Akkarasiritharattana & Chamutpong, 2019), antibacterial (Farzinebrahimi et al., 2016; Napisah et al., 2011), antidiabetic (Ishak et al., 2013) and aphrodisiac (Mohd Jaafar et al., 2017). However, no studies have reported for anti-inflammatory activity in this plant (Abu Bakar et al., 2018). Therefore, further scientific studies should be carried out to look at the effectiveness of *M. latifolia* for inflammation treatment, particularly due to their common use for treating acute inflammatory conditions as aforementioned.

4.6.1.2 *Tacca integrifolia*

Figure 4.10*: Tacca integrifolia* (Photo taken at Kampung Orang Asli Batu 16, Gombak).

Tacca integrifolia is an herbaceous plant that belongs to Taccaceae family. This plant is widely distributed in lowland and hill forests in both Peninsular and East Malaysia. It has long leaves that grow vertically and are short stemmed. *T. integrifolia* has long

whisker-like filiform bracteoles which hang beneath the flowers and can extend up to 30 cm in length (Norzielawati, 2017). The *T. integrifolia* known as black lily has many different vernacular names among Malaysians such as *belimbing tanah*, *keladi murai* and *janggut Adam* while the Temuan people in this study, call it as *bunga tedung* (Figure 4.10).

They use the decoction of *T. integrifolia* leaves for treatment of swelling. Its leaf decoction is taken orally, as well as for bathing. The rhizome is used to treat waist pain. There is no other report on the utilization of this medicinal plant from other Temuan tribes. However, Rachdiati & Zakaria (2018) and Rao et al. (2016) documented the utilization of *T. integrifolia* among Temiar tribes in Perak and Kelantan, respectively. They believed that the leaves of *T. integrifolia* are effective in treating back pain and diabetes. Ismail et al. (2015) reported the utilization of *T. integrifolia* leaves among the Jakun tribes in Johor for joint pain, swelling and sprain treatment. In Malay folk traditional medicine, the plant is also used to control hypertension and treat skin diseases. The roots and leaves of this plant are used as herbal baths for postpartum women to refresh the women's body while the leaves are pounded and applied onto the rashes due to caterpillar stings (Ramli et al., 2015; Sabda, 2013).

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4.6.1.3 *Hymenocallis speciosa*

Figure 4.11: ***Hymenocallis speciosa*** **(Photo taken at Kampung Orang Asli Batu 16, Gombak).**

Hymenocallis speciosa is a perennial herb with greenish leaves locally known as *melong kecil* while the Temuan in this study called it as *kancing suasa*. This plant is widely distributed in China, India, Bangladesh, Thailand, Myanmar and Malaysia. This plant belongs to the family Amaryllidaceae and is used as traditional medicine as well as an ornamental plant (Figure 4.11). According to the respondents in this study, the leaves are heated over the fire, pounded before adding the coconut oil and applied to the swelling area. In some cases, the heated leaves are bound topically to the sprain legs or hand. In contrast, the Temuan in Kampung Ulu Kuang, Gombak took the decocted leaves of *H. specioasa* as herbal baths for jaundice treatment (Azliza et al., 2012).

Therefore, these three selected medicinal plants that are commonly used among *Orang Asli* and other tribes in Malaysia were evaluated for their anti-inflammatory potential to provide *in vitro* evidence of the efficacy of the plants in treating inflammation.

4.6.2 Extraction yield and inhibition of protein denaturation assay

In the present study, the ethanolic and aqueous extracts of selected plants were prepared. The aqueous extracts were prepared to imitate the application by *Orang Asli* as herbal remedies. The efficiency of extraction is influenced by a few factors including methods of extraction, solvents used as well as nature phytochemicals (Azwanida, 2015; Truong et al., 2019). The ethanolic and aqueous extraction yield of *M. latifolia, T. integrifolia* and *H. speciosa* is listed in Table 4.3. There are numerous studies reported that organic solvents have better activity compared to aqueous extracts (Djuichou Nguemnang et al., 2019; Do et al., 2014). In the present study, when aqueous extracts of all the three plants were compared, the extraction yield was maximum in *H. speciosa*. This may be because the phytoconstituents present in this plant is extracted better on application of heat compared to other plants. On the other hand, the results indicate that the highest yield extract of these three medicinal plants obtained by maceration method compared to decoction method with ethanol extract of *M. latifolia* showed the highest yield extract . It varies from plant to plant may be because of the nature of secondary metabolites present in them and also their proportion (Sulaiman et al., 2015).

Table 4.3: Extraction yield of selected medicinal plants

Medicinal plants	Extraction	Dry weight (g)	Dry crude extract (g)	Percentage (%)
M. latifolia	80% Ethanol	45.04	11.57	25.69
	Aqueous	33.75	1.16	3.44
T. integrifolia	80% Ethanol	36.03	7.85	21.79
	Aqueous	37.57	1.18	3.14
H. speciosa	80% Ethanol	43.5	8.52	19.59
	Aqueous	38.14	3.19	8.36

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Hence, this *in vitro* study was conducted to investigate the existence of anti-inflammatory activity in extracts of *M. latifolia*, *T. integrifolia* and *H. speciosa*. To evaluate whether the ethanolic and aqueous extracts of these selected medicinal plants possess anti-inflammatory potential, inhibition of protein denaturation assay was performed based on study done by Bouhlali et al. (2016) with minor modifications, because this method is inexpensive and easier to conduct as well as not involved in the ethical issues of handling animals. An independent-samples t-test was performed to compare the difference percentage inhibition of protein denaturation between ethanolic and aqueous extract for all these three selected plants. The *p*-value less than 0.05, and 0.01 was considered statistically significant.

Indomethacin, a non-steroidal anti-inflammatory drug (NSAIDs) have been used as a standard drug in this assay. Based on the results obtained in this present study as shown in Table 4.4, the standard drug indomethacin indicated 57.14 ± 0.00% inhibition rate at concentration of 100 µg/mL. Meanwhile the highest percentage inhibition against protein denaturation was 69.33 ± 0.00% at 200 µg/mL and exhibited a decreasing trend of inhibition rate between 300 to 500 µg/mL (Figure 4.12). Dharmadeva et al. (2018) was also reported a similar trend of inhibition rate for ibuprofen as a standard drug in inhibition of protein denaturation of *Ficus racemosa* extract. This finding was in contrast with previous studies, reporting the drugs that have been used in evaluation of inhibition of protein denaturation assay that inhibited bovine serum albumin denaturation in a dose-dependent manner such as diclofenac sodium (Agarwal & Shanmugam, 2019; Alamgeer et al., 2017), and aspirin (Ullah et al., 2014; Padmanabhan & Jangle, 2012).

Protein denaturation is associated with the occurrence of the inflammatory response that leads to multiple inflammatory illnesses such as arthritis (Ullah et al., 2014;

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Mizushima, 1964). Application of thermal or external stress can cause protein denaturation where involves the loses of secondary and tertiary structures . The main mechanism of NSAIDs as a protection against protein denaturation is by blocking the activity of the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) from synthesis of prostaglandins (Ullah et al., 2014).

Table 4.4: Effect of indomethacin on protein denaturation.

Concentration (µg/mL)	Percentage inhibition
100	57.14 ± 0.00
200	69.33 ± 0.00
300	16.21 ± 1.82
400	5.26 ± 3.04
500	4.02 ± 1.30

Values are represented by means ± SEM in triplicates

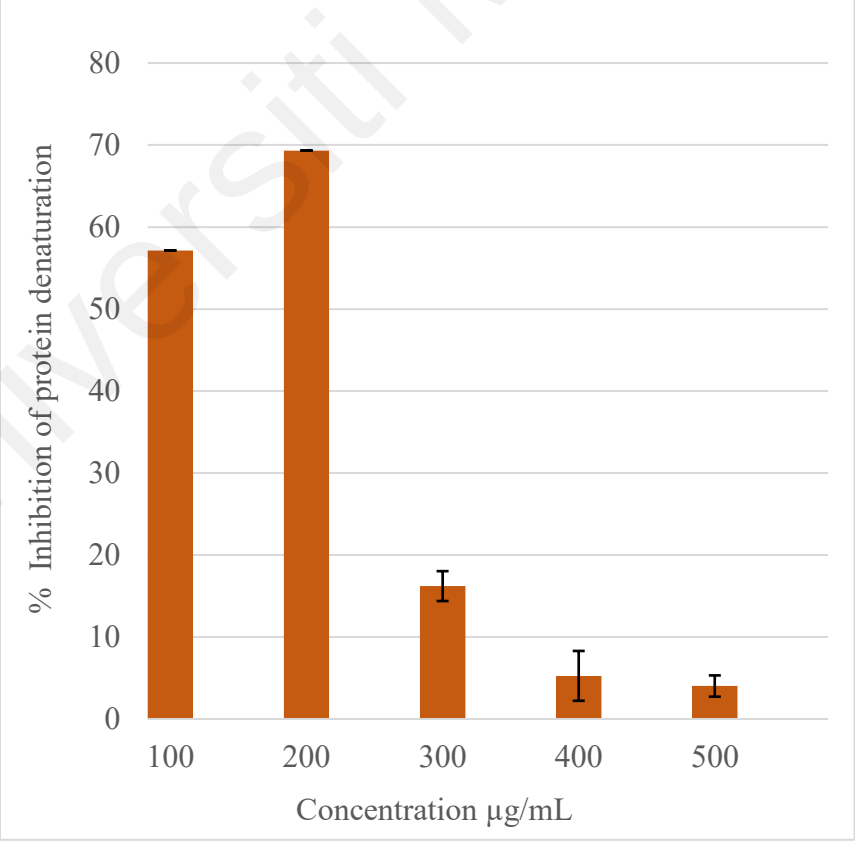

Figure 4.12: Percentage inhibition of protein denaturation of indomethacin. Values are expressed as means ± SEM (n=3).

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4.6.2.1 Inhibitory effects of *Molineria latifolia* extract

The effect of ethanolic extract of *M. latifolia* leaves along with the aqueous extract was evaluated against the denaturation of BSA *in vitro* and results are shown in Table 4.5. Both extracts had a similar trend of inhibition as shown in Figure 4.13. Percentage inhibition of protein denaturation for both ethanol and aqueous extracts of *M. latifolia* was increasing with concentration (100 to 500 µg/mL) within the range from 65.63 ± 1.56% to 155.91 ± 2.15%. *M. latifolia* has a significant difference ($p<0.01$ and $p<0.05$) in inhibition of protein denaturation between ethanol extract compared to aqueous extract for all the concentrations except at concentration 500 µg/mL. In addition, the highest inhibition by *M. latifolia* for both ethanol (87.18 ± 5.13%) and aqueous (71.67 ± 1.67%) extracts were very greater compared to indomethacin that exhibited only 69.33 ± 0.00% inhibition of protein denaturation at concentration of 200 µg/mL.

Various studies have been carried out on antioxidant (Akkarasiritharattana & Chamutpong, 2019), antibacterial (Farzinebrahimi et al., 2016; Napisah et al., 2011), aphrodisiac (Mohd Jaafar et al., 2017), and the antidiabetic activities (Ishak et al., 2013) of the *M. latifolia* (previously known as *Curculigo latifolia*), but to our knowledge this is the first conducted to evaluate the anti-inflammatory potential on this plant species. However, researchers have reported a quite number of studies on the anti-inflammatory activity of other *Curculigo* sp. A previous study done by Asif & Kumar (2010) was evaluated the effects of three different extracts for anti-inflammatory activity using carrageenan-induced paw edema assay in albino rats with indomethacin as a standard drug. Ethanol extract of *Curculigo orchiodes* rhizomes (EE), its alkaloidal fraction (AF), and non-alkaloidal fraction (NAF) were used in this study. Percentage inhibition of paw edema for these extracts was concentration-dependent with the highest percentage inhibition for EE, AF, and NAF were 39.03%, 41.17%, and 37.44% respectively on 500

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mg/kg body weight after 2 hours of carrageenan administration. AF extract possess comparable anti-inflammatory activity of indomethacin (48.66%). However, these three extracts showed significant ($p<0.05$) anti-inflammatory activity in carrageenan-induced rat paw edema when compared to control group. This finding could support the reported traditional usage of *C. latifolia* as it was in the same genus as *C. orchiodes*.

According to Ooi et al. (2018), there were several bioactive compounds found in *C. latifolia* such as cinnamic acid, benzoic acid-derived and flavonoid-derived compounds such as hesperetin, apigenin, dryopteric acid, and isorhamnetin that have antioxidant activity and probably have great effect in anti-inflammatory activity as well. Besides, a previous study was also supported that the ethanolic extract of medicinal plants have higher anti-inflammatory activity compared to water extract. Study done by Djuichou Nguemnang et al. (2019) reported that ethanol extract (44.44%) of *Dissotis thollonii* showed significantly higher ($p<0.001$) inhibition of protein denaturation compared to aqueous extract (42.51%) at a concentration of 1000 µg/mL. This finding is in agreement with the results in the present study.

Therefore, this present study can be used as a preliminary screening, and interestingly the findings support the Temuan people's claims about the existence of anti-inflammatory activity in *M. latifolia*. However, in future, more studies must be conducted to evaluate and determine the active phytoconstituents in *M. latifolia* that responsible for the anti-inflammatory activity. It can provide more evidence that this plant has significant effects on inflammation treatment.

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Table 4.5: Effect of ethanolic and aqueous extract of *Molineria latifolia* on protein denaturation

Concentration (µg/mL)	Percentage inhibition		*P* value
	Maceration (80% Ethanol extract)	Decoction (Aqueous extract)	
100	76.67 ± 3.33*	65.63 ± 1.56	0.04
200	87.18 ± 5.13*	71.67 ± 1.67	0.0452
300	116.00 ± 0.00**	87.50 ± 1.56	0.0001
400	122.58 ± 4.93**	94.52 ± 3.62	0.0101
500	143.86 ± 0.88**	155.91 ± 2.15	0.0066

Values are represented by means ± SEM in triplicates. An independent t-test was performed as the test of significance. Values of *p <0.05 and **p <0.01 were considered significant.

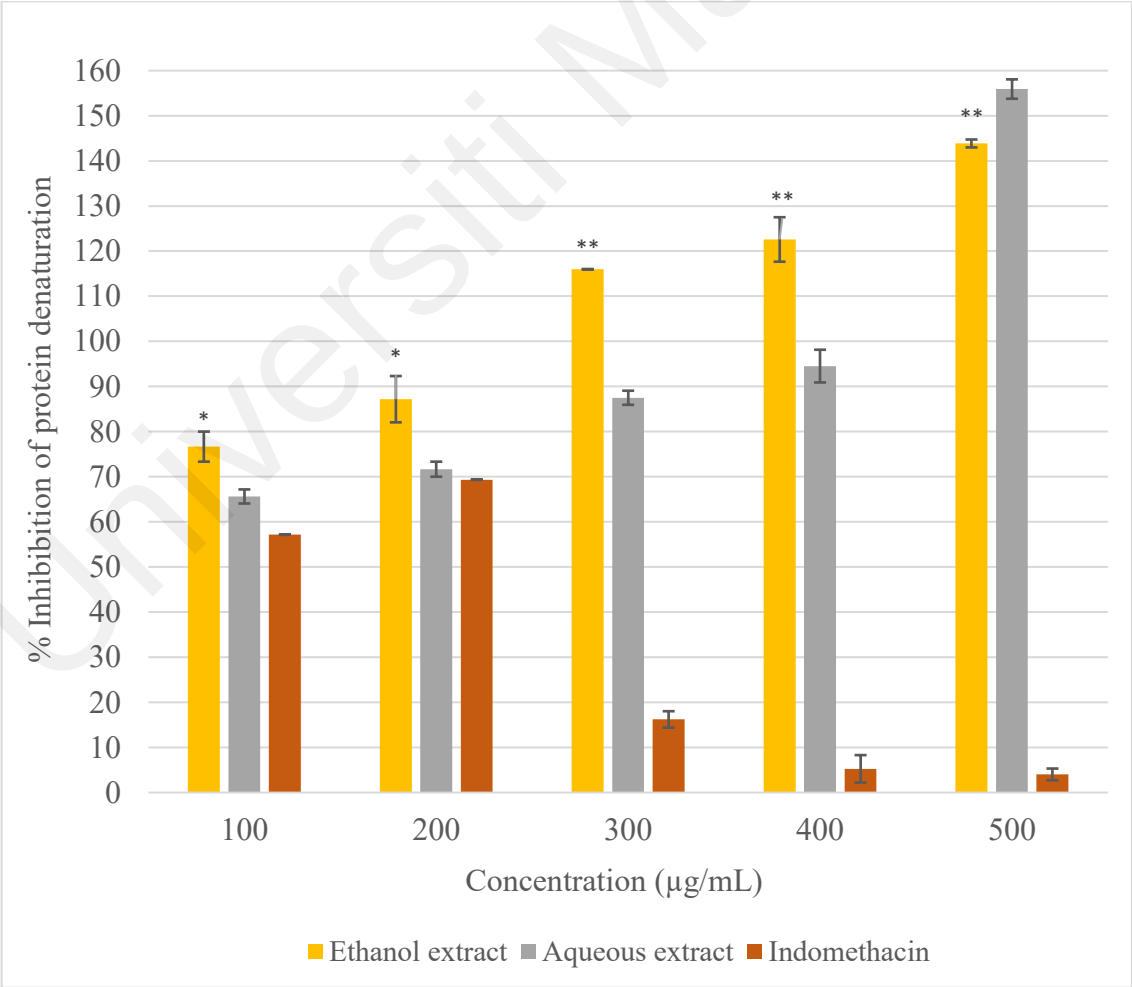

Figure 4.13: Percentage inhibition of protein denaturation of *Molineria latifolia* in comparison to indomethacin. Values are expressed as means ± SEM (n=3).

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4.6.2.2 Inhibitory effects of *Tacca integrifolia* extract

Figure 4.14 illustrates the similar trend of inhibition for both extracts of *T. integrifolia* leaves. Based on the results in Table 4.6, ethanol extract of *T. integrifolia* possessed the highest percentage inhibition of protein denaturation at a concentration of 100 µg/mL with 86.01 ± 4.04% while the aqueous extract had the highest percentage inhibition at a concentration of 300 µg/mL with only 31.88 ± 1.45%. This result showed that inhibition of protein denaturation of *T. integrifolia* extracts was not concentration-dependent. However, there was a significant difference (p<0.01) between these two extracts which are ethanol extract has the higher inhibition of protein denaturation compared to aqueous extract throughout the concentration. This was probably due to greatest content of phenolic compound in the ethanolic extract compared to water extract (Do et al., 2014). Meanwhile, ethanol extract of *T. integrifolia* leaves at a concentration of 100 µg/mL exhibited greater anti-inflammatory activity compared to indomethacin with 69.33 ± 0.00% inhibition of protein denaturation at a concentration of 200 µg/mL.

This finding is supported by Ahmed et al. (2019) who demonstrated that ethanol extract of *T. integrifolia* rhizome possessed significant effects (p<0.01) in reduction of the paw edema in mice induced by carrageenan injection at a dose of 400 mg/kg body weight. 60.9% of paw edema was reduced at the 3rd hour administration of the extract. It was also comparable to the standard drug, diclofenac sodium with 75.60% inhibition at the same period of time. This study may indicate that the efficacy of edema suppression is due to the extract's ability to inhibit the prostaglandin synthesis that involved in inflammation. Carrageenan-induced paw edema assay is a well-known method in evaluating the contribution of mediators involve in vascular changes associated in acute inflammation. The sub-plantar surface of the mice paw that has been injected with carrageenan will induce two phases of edema. The first phase observed at approximately

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1 hour is characterised by the release of histamine, serotonin, and bradykinin. The second phase at 2 to 4 hours, is attributed to neutrophil infiltration and release of prostaglandin (Vinegar, Schreiber & Hugo 1969). This previous study provided evidence to support traditional usage of *T. integrifolia* extract for inflammation treatment since it has promising anti-inflammatory activity.

Futhermore, Autsavakitipong et al. (2015) also reported that ethyl acetate extract of the leaf extract of *T. integrifolia* at the doses of 200 mg/kg significantly inhibited pain caused by acetic acid injection in rats. It was also confirmed by a previous study in mice models that ethanol extract of *T. integrifolia* possessed remarkable analgesic activity in formalin-induced paw licking test at the doses of 200 and 400 mg/kg body weight (Ahmed et al., 2019). In addition, previous studies on *T. integrifolia* also provide evidence on the potential of *T. integrifolia* as an anti-hypertension (Jamaludin & Mohamad, 2016; Kitjaroennirut et al., 2005) as mentioned by the *Orang Asli*.

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Table 4.6: Effect of ethanolic and aqueous extract of *Tacca integrifolia* on protein denaturation.

Concentration (µg/mL)	Percentage inhibition		*P* value
	Maceration (80% Ethanol extract)	Decoction (Aqueous extract)	
100	86.01 ± 4.04**	30.65 ± 1.61	0.0002
200	54.72 ± 3.27**	13.21 ± 3.27	0.0004
300	72.88 ± 1.69**	31.88 ± 1.45	0.0001
400	59.6 ± 5.77**	24.36 ± 1.28	0.004
500	74.75 ± 1.01**	30.48 ± 1.90	0.0001

Values are represented by means ± SEM in triplicates. An independent t-test was performed as the test of significance. Values of $*p < 0.05$ and $**p < 0.01$ were considered significant.

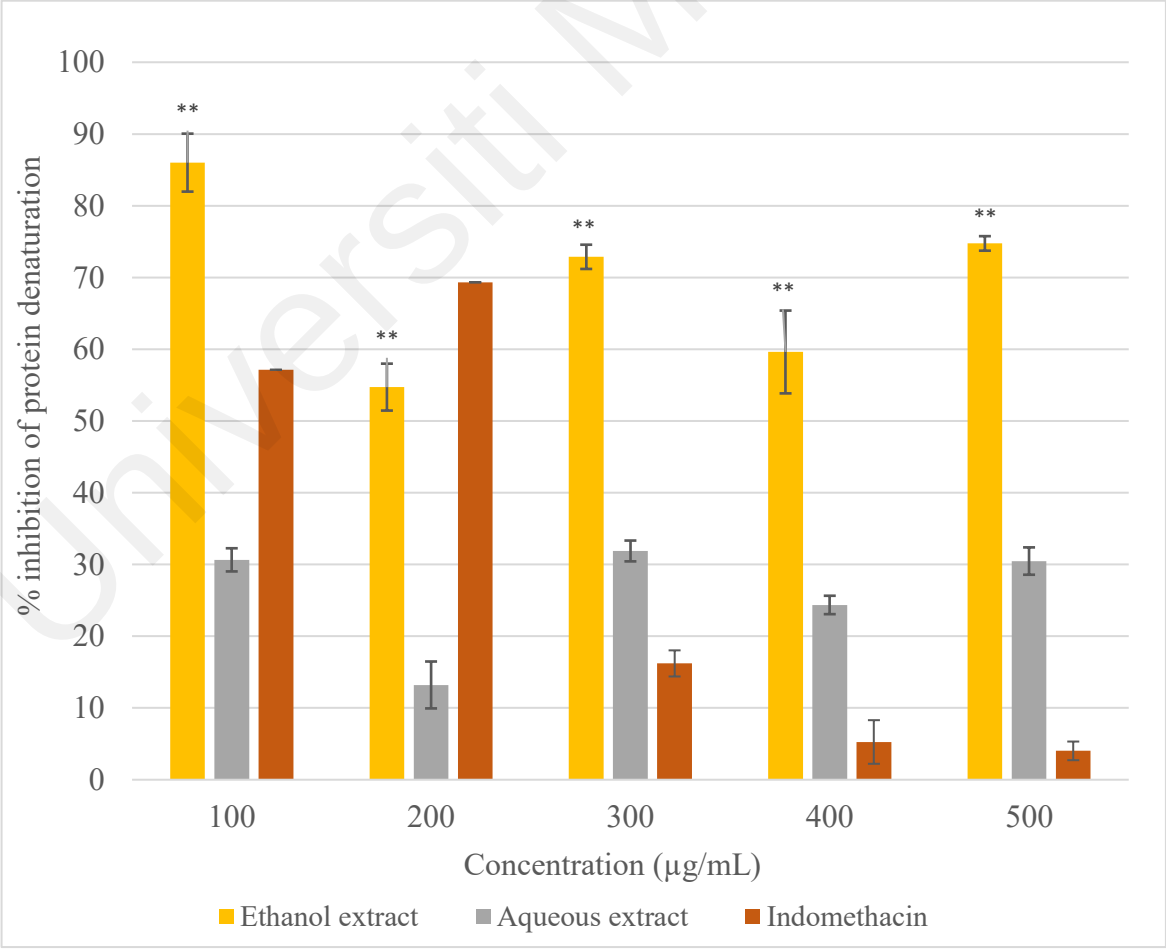

Figure 4.14: Percentage inhibition of protein denaturation of *Tacca integrifolia* in comparison to indomethacin. Values are expressed as means ± SEM (n=3).

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4.6.2.3 Inhibitory effects of *Hymenocallis speciosa* extract

The effects of ethanolic and aqueous extracts of the *H. speciosa* leaves in inhibit the protein denaturation was presented in Table 4.7. *H. speciosa* have a different trend on their protection against denaturation of BSA compared to *M. latifolia* and *T. integrifolia*. Results obtained from the study demonstrate that aqueous extract of *H. speciosa* has good anti-inflammatory activity compared to ethanolic extract at all concentrations as shown in Figure 4.15. The aqueous extract produced the highest inhibition of protein denaturation with 87.18 ± 2.56% at a concentration of 200 µg/mL while only 42.68 ± 0.00% for ethanolic extract in the same concentration. These results have shown that inhibition of protein denaturation is affected by extraction method and there are significant differences (p<0.05 and p<0.01) between aqueous extract and ethanolic extracts. The aqueous extract of *H. speciosa* also exhibited greater suppression of protein denaturation when compared to indomethacin (69.33 ± 0.00%) at a concentration of 200 µg/mL.

According to available literature, there was no study reported on this plant species regarding their anti-inflammatory potential. Nevertheless, Karthikeyan et al. (2016) have conducted an *in vitro* anti-inflammatory assay of *Hymenocallis littoralis* extract by human red blood cell (HRBC) membrane stabilization method. The ethanol extract of *H. littoralis* showed great anti-inflammatory activity at a concentration of 100 µg/mL and 500 µg/mL with 83.46% and 84.72%, respectively. Several biochemical compounds in leaves and flowers extract of *H. littoralis* were successfully determined through phytochemical analysis such as steroid, flavonoids, saponin, carbohydrates, cardiac glycoside, tannins, and phenolics compound (Karthikeyan et al., 2016; Nadaf et al., 2018). In this present study, the extracts of *H. speciosa* exhibit anti-inflammatory activity on suppression of protein denaturation may be due to the presence of similar constituents

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because both plants, *H. littoralis* and *H. speciosa* belong to the same genus and the Amaryllidaceae family.

The aqueous extract of *H. speciosa* showed the highest percentage inhibition of protein denaturation compared to *M. latifolia* extracts and *T. integrifolia* extracts that exhibit good anti-inflammatory activity in their ethanol extracts. These results showed that different plants require different types of solvents to ensure the maximum extraction of the bioactive compounds and the biological activities of the plants. Truong et al. (2019) reported in their study that aqueous extract of *Severinia buxifolia* has higher phenolic compounds being extracted (5.95 mg GAE/g DW) and lower alkaloids and terpenoids meanwhile, ethanol solvent extracted lower phenolic compound (3.60 mg GAE/g DW) and higher alkaloids (1.34 mg AE/g DW) as well as terpenoids (0.97%, w/w) in comparison with distilled water extract (0.16 mg AE/g DW and 0.43%, w/w) respectively.

Hence, the present study on these three medicinal plant extracts exhibited the efficacy against protein denaturation. It could be a potential source of anti-inflammatory drug candidates.

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Table 4.7: Effect of ethanolic and aqueous extract of *Hymenocallis speciosa* on protein denaturation.

Concentration (µg/mL)	Percentage inhibition		P value
	Maceration (80% Ethanol extract)	Decoction (Aqueous extract)	
100	16.67 ± 0.00*	26.67 ± 3.33	0.040
200	42.68 ± 0.00**	87.18 ± 2.56	0.000
300	37.39 ± 1.69**	76.00 ± 0.00	0.000
400	24.15 ± 3.99**	49.46 ± 2.15	0.005
500	5.00 ± 0.00**	22.81 ± 2.32	0.002

Values are represented by means ± SEM in triplicates. An independent t-test was performed as the test of significance. Values of $*p < 0.05$ and $**p < 0.01$ were considered significant.

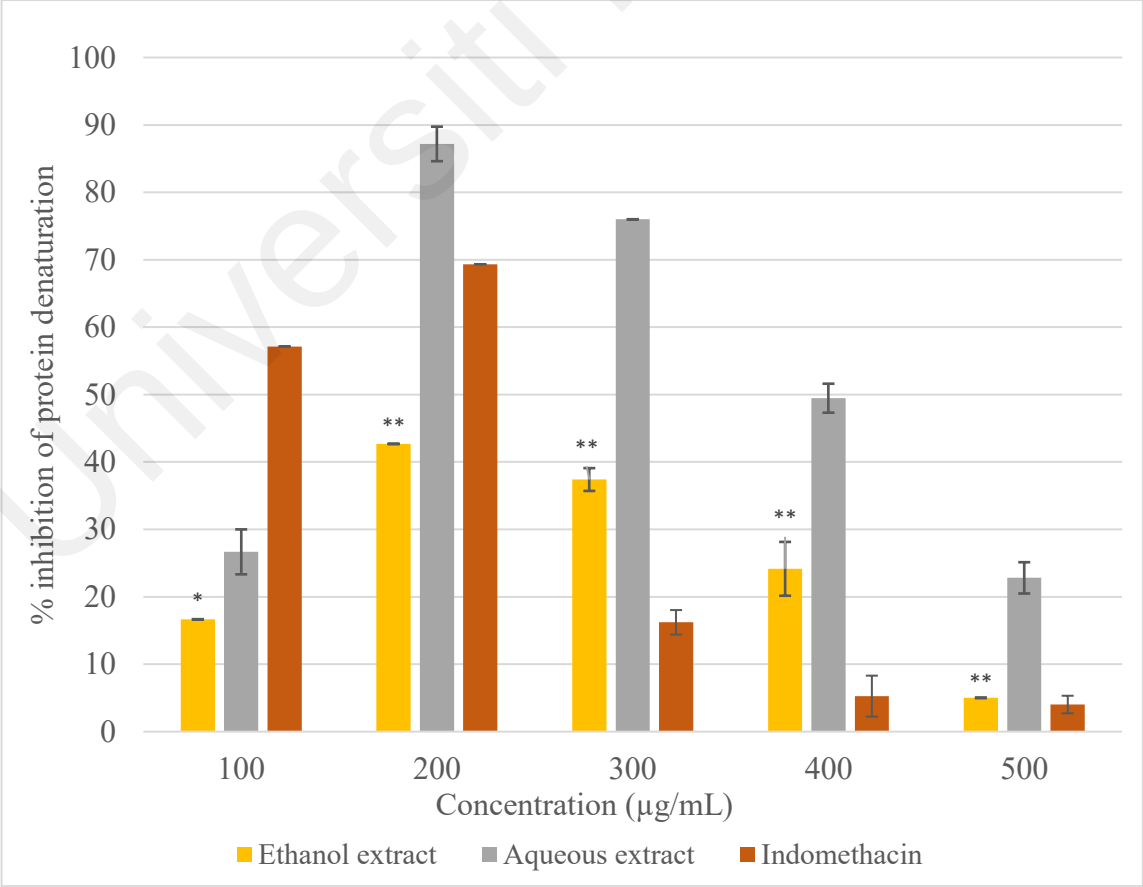

Figure 4.15: Percentage inhibition of protein denaturation of *Hymenocallis speciosa* in comparison to indomethacin. Values are expressed as means ± SEM (n=3).

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CHAPTER 5: CONCLUSION

In this study, the current state of knowledge and application of medicinal plant species for the treatment of various ailments and health conditions among the Temuan tribe in Kampung Orang Asli Batu 16, Gombak, Selangor was successfully documented. A total of 41 medicinal plant species belonging to 28 plant families were reported being used for the treatment of 37 ailments or health conditions. The information of the medicinal plants and their application recorded in this study demonstrate that the Temuan community in Kampung Orang Asli Batu 16, Gombak still possess the traditional knowledge of medicinal plants for the treatment of various ailments and health conditions. This study emphasizes how important it is to document and conserve such traditional knowledge before it becomes depleted as more of the knowledgeable people are the older generation. There is also a clear lack interest among the younger generation on learning traditional knowledge due to availability of modern medicines and socio-economic transformation. The information of this study will also contribute in updating the inventory of existing medicinal plant species in Malaysia and also as references for future studies. These findings also represent useful information to create awareness about the conservation of biodiversity among younger generations.

The *Molineria latifolia*, *Tacca integrifolia,* and *Hymenocallis speciosa*, which are the commonly utilized plants for inflammation treatment among the Temuan in this study, all exhibited the anti-inflammatory activity as shown through the inhibition of protein denaturation assay. Ethanol extracts for both *M. latifolia* and *T. integrifolia* leaves showed higher percentage inhibition of protein denaturation compared to their aqueous extract. Meanwhile, for *H. speciosa*, the aqueous extract exhibited higher rate of inhibition compared to the ethanol extract. The ethanol extract for all these plants indicated a significant difference when compared to aqueous extracts at various concentrations

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within the range of 100 – 500 µg/mL. When compared with the reference drug, indomethacin, all these three selected plants showed comparable anti-inflammatory activity. Therefore, findings of this study support the traditional claims by the Temuan tribe in this study that these plants are effective for treatment of conditions involving inflammation. Anti-inflammatory activity by aqueous extracts also supports the most common mode of preparation used by the Temuan which is decoction.

The findings from the present study could provide baseline information for future research on phytochemical, pharmacological, toxicological, and clinical studies on these plants in order to validate and understand more about the biochemical compounds that involve in the anti-inflammatory activity. It is also to ensure the safety of consuming these medicinal plants as well as for novel drug development. In addition, these findings also would be beneficial to healthcare sector and industry to develop new pharmaceuticals and nutraceuticals especially in the treatment of inflammatory diseases.

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