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Ethnobotanical survey and evaluation of traditional mosquito repellent plants of Dai people in Xishuangbanna, Yunnan Province, China

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ABSTRACT

Ethnopharmacological relevance: Dengue is one of the most important pervasive diseases in many regions of the world, including China. There is an urgent need for new repellents, including plant derivatives, due to the resistance, toxicity, and non-degradability of synthetic insecticides. Traditional plant-based remedies may provide potential avenues for developing new strategies.

Aims of the study: The aims of this study were to 1) document the traditional mosquitoes repellent plants used by the Dai people of Xishuangbanna, China; 2) screen out new efficient mosquito repellent plants as candidates for further study.

Materials and methods: During the period August 2016 to July 2017, five field surveys were conducted in 16 villages of Xishuangbanna. A total of 81 informants (44 males and 37 females) were interviewed using semi-structured questions to collect detailed information on the plants they use to prevent mosquito bites. Ten plants with higher popularity and larger resource were collected and extracts were prepared by hydro-distillation or with petroleum ether. Extracts were tested for adult *Aedes albopictus* repellency using a human-bait cage. Firstly, repellency was determined as the Minimum Effective Dosage (MED) per minute at which 1% of the mosquito bite through the treated cloth. Secondly, five plant extracts with lower MEDs were tested the repellent longevity of different concentrations.

Results: Eighteen plants were documented as being used in traditional remedies against mosquitoes. The methods for controlling mosquitoes were diverse: direct burning was used for most plants (16 species), followed by smearing (5 species), and placing (5 species). Laboratory analyses confirmed that ten plants did exhibit mosquito repellent activity. Of them, *Artemisia indica, Nicotiana tabacum*, *Blumea balsamifera*, *Vitex trifolia*, and *Chromolaena odorata* showed good mosquito repellency with MEDs of 0.015, 0.061, 0.090, 0.090, and 0.105 mg/cm^2, respectively. The protection rate provided by *A. indica* is also the highest among five plants. Although it provides complete protection time of only 30 min at 0.45 mg/cm^2 concentration, its repellency within 2 h is not significantly different from that of DEET.

Conclusion: Dai villagers in Xishuangbanna have a rich, diverse and scientific knowledge of plant-based mosquito repellents. Laboratory experiments screened out several plants as candidates for mosquito repellents, of which *Artemisia indica* was the most promising candidate plant.

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1. Introduction

Dengue—one of the most important pervasive diseases in the world—is a vector-borne viral disease that is transmitted to humans by female *Aedes* mosquitoes. Infection with the dengue virus (DENV) causes a flu-like illness, which occasionally develops into severe dengue, with potentially lethal complications. In recent years, dengue fever has spread rapidly in all regions of the world, and the incidence has recently increased dramatically; there has been a 30-fold increase in reported dengue cases in the past 50 years (WHO, 2012). According to Bhatt et al. (2013), there are approximately 390 million dengue infections each year (95% Confidence interval 2.84–258 million), of which 96 million (0.67–1736 million) manifest clinically, with varying severity. Another study on the prevalence of dengue showed that 3.9 billion people in 128 countries worldwide are at risk from dengue virus infection (Brady et al., 2012). Unfortunately, there is no universal vaccine or specific effective treatment for dengue fever. Thus, vector control is one of most important approaches to tackle dengue fever.

Aedes aegypti (L.) is the primary vector of the dengue virus worldwide. The *Ae. aegypti* mosquito lives in urban habitats and preferentially feeds on humans. *Ae. aegypti* is a daytime feeder and bites multiple people during each feeding period. *Aedes albopictus* (Skuse) (the tiger mosquito) is also a major vector of dengue, second only to *Ae. aegypti* (WHO, 2012). The geographical range of *Ae. albopictus* has been spreading rapidly in the last three decades (Waldock et al., 2013). *Ae. albopictus* originated in tropical and temperate Asia (Paupy et al., 2009), and has now spread to North America and more than 25 countries in Europe, largely due to international trade (WHO, 2012). The main factors contributing to the successful spread of *Ae. albopictus* are thought to be its enormous physiological and ecological plasticity (Paupy et al., 2009). These two *Aedes* mosquitoes are also vectors of other global arboviruses, including yellow fever (Jentes et al., 2011), and chikungunya (Leparc-Goffart et al., 2014). Combined with the potential effects of climate change, it is likely that the future geographical ranges of mosquitoes and diseases will continue to expand if we do not implement effective measures immediately.

Mosquito control interventions proposed by the World Health Organization (WHO) include environmental management of mosquitoes' breeding sites and use of chemical insecticides (WHO, 2012). The elimination of breeding sites is a large and intermittent effort and often ineffective because it is hard to manage all breeding sites (Tavares et al., 2018). Therefore, most control strategies are based on the use of chemical insecticides, such as indoor residual spraying, space sprays, larvicides, and long-lasting insecticidal nets. However, after the application of insecticides for many decades, mosquitoes have developed resistance to many of them (Bisset et al., 2013). In addition, the continuous use of synthetic insecticides causes ecological disturbance by affecting non-target insects (Wasim et al., 2009), and may cause respiratory problems for humans, particularly children (Salameh et al., 2003).

The application of repellents is an effective implement to prevent mosquito bites, as repellents can provide protection anywhere and anytime. *N, N*-diethyl-3-methylbenzamide (DEET) is the most commonly used mosquito repellent and has been considered the most broad-spectrum and efficacious (Nguyen et al., 2018). DEET has been used as a repellent since the 1950s but tolerance to DEET in mosquitoes has been reported (Brown and Hebert, 1997). DEET may degrade vinyl and plastics and harm some synthetic clothes such as rayon, pigmented leather, and spandex (Brown and Hebert, 1997). Overuse of DEET can also cause neurotoxicity, dermatitis, and allergic reactions (Antwi et al., 2008). In addition, the high cost of DEET and other synthetic mosquito repellents is unaffordable for many people in rural areas of countries with mosquito-borne diseases. As a result of these issues, there is an urgent need to screen new repellents, especially plant derivatives. Plant derivatives are considered to be aromatic, safe, broad-spectrum, and they degrade easily (Belmain et al., 2001; Das et al., 2003, 2015; Isman, 2006; Sharma et al., 2004).

Traditional remedies against mosquitoes provide potential avenues for developing new strategies. Records of human use of plant repellents can be traced back to 484–425 years BC (Moore and Debboun, 2006). Due to the ease of obtaining materials, simple procedures, and low economic cost, some people in rural areas still use these traditional methods to control mosquitoes. Ethnobotanists have conducted surveys of traditional insect repellents in various regions, including Cameroon (Youmsi et al., 2017), China (Fan et al., 2019; Moore, 2005), Eritrea (Waka et al., 2004), Ethiopia (Karunamoorthi et al., 2009; Kidane et al., 2013), Guinea Bissau (Pålsson and Jaenson, 1999), Iran (Cheraghi Niroumand et al., 2016), Italy (Guarrera, 1999), Kenya (Gakuya et al., 2013), Laos (De Boer et al., 2010), Sierra Leone (Kanteh and Norman, 2015), South Africa (Mavundza et al., 2011), Spain (González et al., 2011), Tanzania (Innocent et al., 2014; Kweka et al., 2008), and Uganda (Mwine et al., 2011). A number of ethnomedicinal surveys also reported traditional insect repellents (Duke, 1975; Packer et al., 2012; Rahmatullah et al., 2010). Laboratory experiments have also verified that a number of traditional mosquito repellent plants do have good repellent or insecticidal activity against mosquitoes. For example, Kweka et al. (2008) tested two of the most commonly used insect repellent plants in the northwestern part of Tanzania, namely *Ocimum kilimandscharicum* Gürke and *Ocimum gratissimum* L. Arm-in-cage tests using these two plants showed 83% and 91% efficacy compared with DEET, respectively. Mavundza et al. (2014) evaluated the adulticidal activity of 10 plants traditionally used as mosquito repellents in South Africa. Dichloromethane and ethanol extracts of *Aloe ferox* Mill. leaves showed 98% and 86% adult *Anopheles arabiensis* Patton mortality, respectively. As a part of the unique knowledge and traditions of the native inhabitants, more knowledge surveys of traditional mosquito repellent plants are needed to provide diverse directions and materials for the development of plant-based mosquito repellent products.

The Dai people, one of the major populations in Xishuangbanna, China, have lived in the rainforest for generations. They have accumulated a rich and diverse knowledge of plants (Liu et al., 2002; Liu et al., 2002; Pan et al., 2006). Thus, the first objective of this study was to document the traditional plant-based mosquitoes repellent remedies of the Dai people of Xishuangbanna. The dissemination of results to the community may provide acceptable, convenient, and inexpensive mosquito repellents for underdeveloped areas. The second aim was to evaluate repellent activities of traditional preferred plants against *Ae. albopictus* under laboratory conditions and try to screen out new efficient mosquito repellent plants as candidates for further study.

2. Materials and Methods

2.1. Study area

Xishuangbanna (21°08′ to 22°36′ N, 99°56′ to 101°50′ E) is an autonomous prefecture of Yunnan Province, located in southeast China (Fig. 1). This prefecture has a 966.3 km border with Laos and Myanmar and an area of 19124.5 sq km. The climate in Xishuangbanna is warm and humid throughout the year: in 2018, annual rainfall was between 1347.4 and 1916.8 mm, and the annual average temperature is between 18.6 and 22.9 °C (Office of the Government of Xishuangbanna Dai Autonomous Prefecture, 2019). Annual solar radiation in the region is 116.724 kcal/cm^2/year, and annual sunshine averages 1858.7 h (Cao et al., 2006). The topography consists of alternating valleys and hills with elevations ranging from 2429 m in the north to 477 m in the south. The valleys have a tropical monsoon climate and the hills are subtropical (Xu et al., 2005).

Xishuangbanna is included in the Indo-Burma biodiversity hotspot and has a rich diversity of flora and fauna (Myers et al., 2000). The climate of Xishuangbanna is suitable for mosquitoes and insect breeding. There are 260 species of mosquito from 19 genera in the Xishuangbanna area, accounting for 89.35% of the species of Yunnan Province (Dong

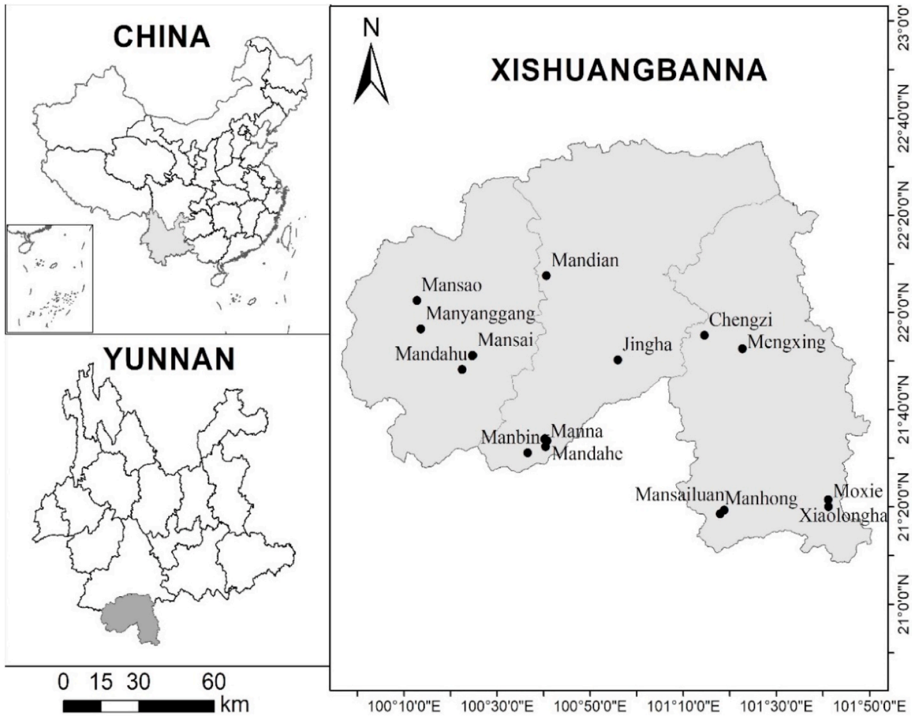

Fig. 1. Sites of ethnobotanical surveys of plant-based remedies against insects: 16 Dai villages with herbalists were identified using snowball methods through the recommendations of Dai people in Xishuangbanna.

et al., 2005). Mosquito bites bring not only discomfort but also the risk of many diseases. Xishuangbanna was historically a high-risk area for malaria, however, by the end of the 20th century, the prevalence of malaria in this area was low (Li et al., 2012). In recent years, imported dengue cases have become common in Xishuangbanna as it is located on the southwestern border of China and is adjacent to dengue-affected areas in Laos and Myanmar. *Ae. albopictus* is the main vector of dengue fever in Xishuangbanna (Li et al., 2016). The first outbreak was reported in 2013, with 1245 confirmed DENV-3 cases (Zhang et al., 2014). The results of phylogenetic analyses suggested that the Yunnan isolates may have originated from neighbouring countries (Zhang et al., 2014). In 2015, 1132 confirmed DENV-2 cases were reported in Xishuangbanna (Zhao et al., 2016). In May 2017, Xishuangbanna had its first major DENV-1 outbreak when DENV also broke out in Myanmar (Lin et al., 2019). Controlling vector-borne diseases and preventing mosquito and insect bites is a vital issue for local governments and residents.

There are 13 ethnic groups living in Xishuangbanna. The Dai people have the largest population, accounting for about one-third of the population of the whole prefecture. The Dai people are mainly distributed in valleys along the Lancang River and its tributaries. They have lived in a self-sufficient agricultural natural economic society for generations and have a high level of understanding and utilization of plants. Dai medicine (one of China's "Four Major National Medicines") is a unique and complete system. With the development of modern society, knowledge of insect repelling plants has been gradually lost with the use of modern products. There are only a small number of people who still retain knowledge of traditional insect repellents, usually herbalists. The existence of a herbalist in a village affects the diversity of traditional insect repellent knowledge of the villagers. Therefore, we selected 16 Dai villages with herbalists as study sites identified by snowball sampling of Dai people in Xishuangbanna (as shown in Fig. 1).

2.2. Ethnobotanical data collection

In order to obtain local information about traditional uses of mosquito repellent plants in Xishuangbanna, five field surveys were conducted between August 2016 and July 2017. We were accompanied by local people as native guides and translators for all our visits. Meetings with village administrators to inform them of the purpose of our work were conducted prior to the surveys. The village head would consent to the research and recommend traditional healers or herbalists to be interviewed for the study. In addition, we interviewed elderly villagers who were known to be knowledgeable about plants by the local community. As shown in Table 1, a total of 81 informants (44 male and 37 female) were identified as having good knowledge of insect repellent plants. The ages of informants ranged from 30 to 92 years old, with an average of 62 years old. Most informants were between 50 and 69 years old. There were 18 herbalists, 58 ordinary residents that depend on agriculture, and 5 village officials.

Informants were interviewed to document detailed information on the plants they use to repel mosquitoes, including vernacular names, plant parts used, method of preparation, method of administration, and any other information that was offered. Plants were collected and identified by herbalists with the assistance of researchers during transect walks. Plants were photographed at their locality, collected, and later identified by researchers. Voucher specimens were deposited in the

Table 1
Gender, age, and occupation of interviewees.

	Number	Percentage
Gender		
Male	44	54.3%
Female	37	45.7%
Age		
30–50	7	8.6%
50–59	25	30.9%
60–69	24	29.6%
70–79	18	22.2%
>79	7	8.6%
Occupation		
Herbalist	18	21.0%
Farmer	58	71.6%
Others	5	7.4%

herbarium of the Kunming Institute of Botany, China (KUN). Plant species were cross-checked via The Plant List (www.theplantlist.org) for the accepted name of the species.

For each cited plant species, the number of informants that referred to each usage was counted as the Frequency of Citation (FC).

2.3. Plant material collection and extraction

Ten plants with higher popularity and larger resource were selected for repellence testing. These plants were collected by researchers in the field. The specific details of collection and extraction are shown in Table 2. Plant materials were dried under shade at room temperature (18–23 °C) and then powdered. Most of these plants were hydro-distilled for 3 h using the standard apparatus described in the Chinese Pharmacopeia (State Pharmacopedia Commission, 2015). Plant material from *Clerodendrum bungei* and *Nicotiana tabacum* was extracted with petroleum ether because the essential oil yield from these plants is very low. Two hundred grams of plant material was dissolved in 2 L of petroleum ether for 24 h, and then vacuum rotatory evaporator was used for the concentration. All extracts were obtained and stored in brown glass bottles under refrigeration at 4 °C for later use.

2.4. Rearing of *Ae. albopictus* mosquitoes

All mosquitoes were reared at the Kunming City Centre for Disease Control and Prevention (Kunming, China). The *Ae. albopictus* colony was maintained under laboratory conditions. In short, a piece of filter paper with eggs was soaked in a flask filled with distilled water containing larval diet [2% slurry of rat feed (Specialty Feeds Pty Ltd., Perth, Australia)]. Eggs were hatched in a culture room at a temperature of 26 ± 1 °C and photoperiod of 12:12 h light: dark. Pupae were transferred to a separate jar with distilled water that was placed in a mesh cage till the adults emerged. Mosquitoes were maintained in the culture room at 26 ± 1 °C and 60 ± 10% relatively humidity under a photoperiod 12:12 h light:dark. Adult females aged 5–9 days were selected for the mosquito repellent bioassays.

2.5. Mosquito repellent bioassay

The repellent activity of plant extractions against *Ae. albopictus* was investigated using the human bait technique. Approximately 400 (±10%) mosquitoes were selected and put into a mesh cage (45 × 35 × 37.5 cm) and held for 30 (±2.5) min before testing. Firstly, repellency was determined as the Minimum Effective Dosage (MED) per minute at which 1% of the mosquito bite or land on the treated cloth. Secondly, five plant extracts with lower MED were tested the repellent longevity of different concentrations. There were two male and two female volunteers in the assay, and all volunteers were informed of the methodology, probable discomfort, and remedial arrangements. They all signed a written informed consent. The protocol was approved by the Kunming Centre for Disease Control and Prevention (NO. 172501300399–11.10).

Table 2
Details of collection, extract forms and yield values of plant extracts.

Plant	Collected part	Location	Extraction	Yield/‰
Ageratum conyzoides	Arial part	21°21′29″N, 100°41′09″E	Essential oil	2.95
Artemisia indica	Arial part	21°53′10″N, 100°15′02″E	Essential oil	3.86
Blumea balsamifera	Leaves	21°53′46″N, 100°14′57″E	Essential oil	5.31
Chromolaena odorata	Arial part	21°55′18″N, 100°14′34″E	Essential oil	1.26
Clausena excavate	Branches	21°53′10″N, 100°15′02″E	Essential oil	2.44
Clerodendrum bungei	Leaves	21°21′29″N, 100°41′09″E	Petroleum ether extract	7.45
Ageratina adenophora	Arial part	21°53′10″N, 100°15′02″E	Essential oil	7.52
Laggera pterodonta	Leaves	21°55′18″N, 100°14′34″E	Essential oil	2.84
Nicotiana tabacum	Leaves	21°21′29″N, 100°41′09″E	Petroleum ether extract	31.6
Vitex trifolia	Branches	21°55′18″N, 100°14′34″E	Essential oil	1.35

2.5.1. Minimum effective dosage test

The experimental design was slightly modified according to Tabanca et al. (2013) and Ma et al. (2019). Test plant essential oils were weighed in vials containing 2 mL of ethanol as solvent, while petroleum ether extracts were dissolved in petroleum ether. Next, 1 mL of the solution was removed to produce an initial concentration on the cloth of 1.5 mg/cm^2. Serial dilutions were then performed to produce corresponding concentrations (i.e. 1.5, 0.75, 0.375, 0.187, 0.094, 0.047, 0.023, 0.011, and 0.005 mg/cm^2) on the cloth using the remaining 1 mL solution. The same dosage of DEET was used as a positive control. Ethanol and petroleum ether were used as the solvent controls. The treated cloth was allowed to dry for 3–5 min before the bioassay was carried out.

The hand and arm of each volunteer was covered with a long rubber glove (Cleanwrap, Korea). An opening (4 × 8 cm) was then cut between the wrist and elbow in order to let odours out and attract mosquitoes. During the trial, the open area was covered with dried sample-treated cotton cloth (5 × 10 cm). At the beginning of the trial, the volunteers placed their arms covered with solvent-treated cloth into the mosquito cage. If there were no fewer than 15 mosquito bites in 1 min, the mosquitoes and volunteers were deemed to qualify for testing. Zero to three bites or landing on treated cloth per minute was denoted as a pass for a sample concentration, lower treatment concentrations were then assessed until one failed. If a concentration of 1.5 mg/cm^2 on cloth resulted in failure, the MED was then recorded as ineffective at this highest concentration. The tested mosquitoes were allowed a 30 min recovery time after 10 successive tests.

2.5.2. Repellent longevity bioassay

Five plant extracts with lower MED were tested for repellent longevity. The evaluation method used was similar to that described by Wu et al. (2019). Plant extracts were diluted and daubed on the skin of volunteers with three concentrations (0.15, 0.30, and 0.45 mg/cm^2). A 5 × 5 cm skin area of each test personnel's hand was daubed, of which 4 × 4 cm was exposed to mosquitoes. The rest of the hand was shielded. The same surface of the other hand was exposed for blank control. After treatment, the volunteer introduced his hand every 30 min into the mosquito cage, and exposed the hand there for 2 min. Before the start of each exposure period, the mosquitoes were tested for their readiness to bite by placing the blank hand. The number of landing mosquitoes at the marked area was recorded at each test. The percentage of repellency was calculated as:

$$\%\ \text{Repellency} = \frac{C - T}{C} \times 100$$

In this formula C and T are the total number of mosquitoes landing and/or biting at the control and treated area, respectively.

2.6. Statistical analysis

Comparison of repellency for each time interval derived from the different test repellent against *Ae. albopictus* were employed by SPSS 20 software (SPSS Inc., Chicago, IL, USA), one-way ANOVA, and Duncan's multiple range test. All differences were considered significant at $p < 0.05$.

2.7. Literature review

Secondary data collection on plants mentioned by informants of Xishuangbanna was done by using different search engines such as Google Scholar, Web of Science and CNKI (China National Knowledge Infrastructure). We tried to identify scientific publications describing related ethnobotanical and laboratory studies of these plants. Bioactive components relevant to mosquito repellent were also reviewed. We combined the following keywords using the Boolean operators 'AND' and 'OR': 'plant name' (e.g. *Artemisia indica*); 'mosquito repellent'; 'essential oil'; 'chemical compound' (e.g. thymol); 'volatile compound'; 'insecticide'.

3. Results and discussion

3.1. Traditional knowledge of plants used as mosquito repellent

Through ethnobotanical surveys, we collected a variety of plants and practices to prevent mosquito bites. A total of 18 plant species belong to 18 genera and 9 families were reported to be used as insect control remedies in the targeted communities (Table 3). The most widely used families are Compositae (6 species) and Lamiaceae (3 species). Seven of these plants were cultivated or semi-cultivated: *Curcuma longa*, *Datura metel*, *Lantana camara*, *Nicotiana tabacum*, *Microtoena patchoulii*, *Vitex trifolia*, and *Toona sinensis*. These plants were not only be used as mosquito repellents, but also medicines, spices, economic and ornamental plants in the daily life of the Dai people. *Datura metel* and *Microtoena patchoulii* were cultivated by herbalists in garden for easy access due to the gradual reduction of wild resources. The reduction was the result of destruction of forests and the lifestyle changes of the Dai villagers in recent years. Most of the other wild plants were common weeds on the roadside or abundant plants, which were convenient to obtain from nature. Most species (68.2%) were herbaceous plants, followed by shrubs (18.2%), trees (9.1%), and lianas (4.5%). Herbs have a shorter life history, wider distribution, and are more abundant than other types of plants, which is useful for acquisition and large-scale utilization. Leaves were the most common plant part (72.7%) used, having the greatest biomass and being the most accessible parts of the plant. Other plant parts used were branches (13.6%), fruits (4.5%), bark (4.5%), rhizomes (4.5%), and the whole plant (9.1%). The preferences of respondents in their selection and use of plants indicated that traditional mosquito repellent plants are both easily accessible and affordable, which is similar to other areas of the world (Innocent et al., 2014; Karunamoorthi et al., 2009).

Compared with other ethnobotanical surveys of mosquito repellent plant in adjacent areas, plant species mentioned are significantly different. Moore (2005) conducted a survey of mosquito repellent plants in villages of 25 ethnic groups in Yunnan province, including the Dai people in Xishuangbanna. Only 3 (out of 20) plants were reported in the results of that study (Moore, 2005). Twenty-four plants were collected from the Hani people in Xishuangbanna (Fan et al., 2019), and there were seven plants used by both Dai and Hani villagers. De Boer et al. (2010) recorded 92 plant species as traditional repellents and insecticides in Laos, and only three of them were also mentioned in current study. Pavela and Benelli (2016) reviewed ethnobotanical knowledge on mosquito repellents reported from ten areas of the Africa region. Native inhabitants of African regions traditionally use 64 plants (30 families), of which only 17 plant species were used by residents of two or more area.

3.2. Modes of administration of repellents

This study recorded a number of modes of administration of plants, including burning (16 species), smearing (5 species), and placing (5 species). The Xishuangbanna area has mosquitoes year-round. When there are a large number of mosquitoes, the Dai villagers bundle the fresh of semi-dried mosquito repellent plants with other flammable firewood into a bundle and ignite them in a room; the smoke produced helps drive mosquitoes away. When they are outdoors, they will cover a lot of fresh plants on the fire. When there are few mosquitoes, *Artemisia indica* is placed directly in the home. Dai villagers also carry a lighted cloth braid with them when they work in the fields. The burning braid is more effective in preventing mosquito bites when it is sprinkled with dried *Microtoena patchoulii* powder. The fresh plants are also smashed

Table 3
Plants reported as mosquito repellents and their uses in Dai villages of Xishuangbanna (China). FC, frequency of citation.

Scientific name (Voucher number)	Family	Vernacular name	Origin	Habit	Parts used	Method of application	FC
Ageratina adenophora (Spreng.) R.M.King & H.Rob (17GY027)	Compositae	Ya-gong-chan	Wild	Herb	Leaves	Burned for fumigation	3
Ageratum conyzoides (L.) L. (17GY006)	Compositae	Ya-e	Wild	Herb	Leaves	Burned for fumigation; Crushed and applied on skin	4
Artemisia indica Willd. (17GY002)	Compositae	Ya-ming	Wild	Herb	Leaves	Burned for fumigation; Crushed and applied on skin; Placed on the floor indoor	20
Blumea balsamifera (L.) DC. (17GY020)	Compositae	Nuo-long	Wild	Herb	Leaves	Crushed and applied on skin	3
Chromolaena odorata (L.) R.M.King & H.Rob. (17GY014)	Compositae	Ya-meng-kam	Wild	Herb	Branches	Burned for fumigation	6
Clausena excavata Burm.f. (17GY025)	Rutaceae	Guo-pie-Fan-nan	Wild	Shrub	Branches	Taken in pocket; Burned for fumigation	5
Clerodendrum bungei Steud. (17GY004)	Lamiaceae	Guo-bin	Wild	Shrub	Leaves	Burned after drying	8
Curcuma longa L. (17GY003)	Zingiberaceae	Hao-min	Cultivated	Herb	Rhizome	Crushed and applied on skin	2
Datura metel L. (17GY034)	Solanaceae	Mu-he-ba	Semi-Cultivated	Herb	Leaves	Burned for fumigation	2
Dysphania ambrosioides (L.) Mosyakin & Clemants (17GY009)	Amaranthaceae	Ya-luo-guo	Wild	Herb	Branches	Burned for fumigation	2
Laggera pterodonta (DC.) Sch.Bip. ex Oliv. (17GY001)	Compositae	Nuo-niu	Wild	Herb	Leaves	Crushed and applied on skin; Burned for fumigation	9
Lantana camara L. (17GY029)	Verbenaceae	Luo-ya-min	Cultivated	Herb	Leaves	Burned for fumigation	3
Litsea cubeba (Lour.) Pers. (17GY030)	Lauraceae	Guo-sha-hai-teng	Wild	Tree	Leaves, Fruits	Burned for fumigation	2
Melia azedarach L. (17GY031)	Meliaceae	Guo-heng	Wild	Tree	Leaves	Burned for fumigation	1
Microtoena patchoulii (C.B.Clarke ex Hook.f.) C.Y.Wu & S.J.Hsuan (17GY018)	Lamiaceae	Ya-huang-beng	Cultivated	Herb	Leaves	Burned for fumigation; Crushed and applied on skin	3
Nicotiana tabacum L. (17GY021)	Solanaceae	Ya-liang	Cultivated	Herb	Leaves	Burned for fumigation	17
Toona sinensis (Juss.) M.Roem. (17GY028)	Meliaceae	Yong-kin	Cultivated	Tree	Leaves	Burned for fumigation	1
Vitex trifolia L. (17GY005)	Lamiaceae	Guan-di	Cultivated	Shrub	Leaves	Burned for fumigation	4

and smeared on the skin, or *Clausena excavate* is put directly in pockets.

Although mosquito repellent plant species reported are different, the traditional modes of administration of various ethnic groups and regions showed great similarity. Smoke and/or thermal expulsions, hanging, and applying juice from fresh plant are three major methods of repelling mosquitoes throughout the world (De Boer et al., 2010; Fan et al., 2019; González et al., 2011; Guarrera, 1999; Karunamoorthi et al., 2009; Mavundza et al., 2011; Youmsi et al., 2017). Burning charcoal alone showed repellent to mosquitoes in a field trial in Bolivia, and the addition of plant material further increased the protection (Moore, 2005). Smoke from a number of traditional indigenous plants showed potent repellent effect against mosquitoes in laboratory, including *Corymbia citriodora* (Hook.) K.D.Hill & L.A.S.Johnson (53.1–72.9%) (Dugassa et al., 2009; Seyoum et al., 2002), *Daniellia oliveri* L. (74.7–77.9%) (Pålsson and Jaenson, 1999), *Olea europaea* L. (79.8%) (Karunamoorthi et al., 2008), *Ocimum basilicum* L. (70.0–73.1%) (Dugassa et al., 2009), and *Ocimum gratissimum* L. (69.6–71.1%) (Dugassa et al., 2009). The repellent effects of release of volatile material through thermal expulsion is better than direct burning in filed and semi-field trials (Dugassa et al., 2009; Seyoum et al., 2002). For example, repellency of *C. citriodora* was 74.5% by thermal expulsion, while it was 51.3% by direct burning in the same trial (Seyoum et al., 2002). Fresh leaves and shoots of *Ocimum forskolei* Benth. in room without processing was tested in Eritrea against *An. arabiensis* and 53% reduction was achieved (Waka et al., 2004). The leaves of *Hyptis suaveolens* (L.) Poit. and *Ocimum americanum* L. provided 73.2 and 63.6% protection respectively, from mosquito bites when hung fresh in the homes in Guinea Bissau (Pålsson and Jaenson, 1999). Crushing and applying juice from fresh plant is the process of distributing plant ingredients on the skin to form a barrier. However, we found no scientific trials on the effectiveness of this usage.

The distinct odour of plant is responsible for the repellent effect when plant is hung or placed in the house without processing. The odour is formed by the volatile organic compounds produced as secondary metabolites (Nerio et al., 2010). Thermal expulsion means that fresh plant material is put on the heat source, so that the volatile is continuously and massively released. The burning of plant materials is a complicated process. Smoke is derived from the thermal degradation and oxidation of the constituents of biomass-derived materials, such as carbohydrates (cellulose and hemicelluloses), lignin, and extractives. This oxidation produces water vapor, permanent gases, volatile organic compounds, semi-volatile organic compounds and particles (Barboni et al., 2010). Particles and gases may mask human kairomones, particularly carbon dioxide, from mosquito searching (Moore, 2005). However, they contribute to air pollution and can be dangerous for human safety and health (Biran et al., 2007). A part of effective volatiles may be oxidized or destroyed at high temperatures, and the newly generated volatile substances from burning showed slight repellent effect, as the protection provided by direct burning is fewer than thermal expulsion of the same plant (Dugassa et al., 2009; Seyoum et al., 2002). This may indicate that volatile organic compounds are responsible for the repellent effect of the traditional remedies. Essential oils are the complex mixture of violate organic compounds isolated from plant materials, and had more promising repellent activity than did extracts using solvents such as hexane, ethyl acetate, and water (Tabacan et al., 2016).

3.3. Documentary information on ethnobotanical status and scientific investigations on the plants mentioned

Secondary data on plants mentioned by Dai villagers in Xishuangbanna was collected by using different search engines (shown on Table 4). Most of these plants were used as repellent target a variety of insects, not only for mosquitoes. Except for *Toona sinensis,* all plants mentioned have been reported to be used traditionally elsewhere to repel mosquitoes or other insects. This study is also the first to report traditional use of *Datura metel* and *Microtoena patchouli* as mosquito repellent. The mosquito repellent activity of nine plants has been reported in previous studies. Repellent efficacy of the same plant varies for different mosquitoes and different laboratory conditions. When tested against *Ae. aegypti*, *Ae. albopictus*, *Anopheles dirus* and *Culex quinquefasciatus* under laboratory conditions, the repellency of essential oil of *Litsea cubeba* at 0.33 μL/cm^2 were 1.8, 6.3, 8.0 and 7.2 h, respectively (Tawatsin et al., 2006). When tested against *Ae. albopictus* using Y-tube olfactometer, *L. cubeba* oil showed 73.94% repellency at 20 min (Wu et al., 2019). However, Trongtokit et al. (2005) observed on repellent of *L. cubeba* against *Ae. aegypti*. Essential oils of all plants contain major compounds that have been identified to have mosquito repellent activity. Active compounds as mosquito repellent are mainly monoterpenoids, sesquiterpenes, diterpenoids and alcohols. In addition, a number of other main components of essential oils have been reported to be insect repellent or insecticide, including (Z)-ascaridole, isoeugenol and tumerone (Chu, 2011; Huang et al., 2002; Zhu et al., 2012). These compounds may also have the effect of repelling or poisoning mosquitoes, which is deserved to research. Since the plants mentioned in this study are mostly widely distributed, there may be multiple chemotypes. Therefore, it is necessary to further analyze the repellent activity and active ingredients of plants reported and collected locally in future research.

3.4. Screening of promising mosquito repellent plants

In this study, 10 selected plants with higher FC and larger resource were tested for their repellence of female *Ae. albopictus* mosquitoes. Most of the test plants were extracted by hydro-distillation to get essential oils. *Clerodendrum bungei* and *Nicousingtiana tabacum* was extracted with petroleum ether because the essential oil yield from these plants is very low. All samples showed a certain level of mosquito repellency, and verify the folk usages of these plants (Table 5). *Artemisia indica* showed the strongest repellency with a MED of 0.015 ± 0.005 mg/cm^2, compared with DEET at 0.019 ± 0.006 mg/cm^2. *N. tabacum*, *B. balsamifera*, *V. trifolia* and *C. odorata* also showed good mosquito repellency, with MEDs of 0.061 ± 0.020, 0.090 ± 0.000, 0.090 ± 0.000 mg/cm^2, and 0.109 ± 0.132 mg/cm^2 respectively.

A lower MED may indicate that the extract contains compounds and/or combination with great repellent activity. However, plant extracts usually cannot provide long time protection, due to their volatility. The repellency rates of five plant extracts with lower MED over time are shown in Table 6. Among the three concentrations tested, the optimal concentration of *A. indica* extract was 0.45 mg/cm^2, while the other four extracts were all 0.3 mg/cm^2. The protection rate provided by *A. indica* is also the highest among five plants. Although it provides complete protection time of only 30 min at 0.45 mg/cm^2 concentration, its repellency within 2 h is not significantly different from that of DEET. *C. odorata* also showed good repellency (>90%) within 1 h at 0.3 mg/cm^2. The repellency percentage is not completely consistent with the MED value. MED of *N. tabacum* extract ranks second, but its repellency rate is the lowest. *C. odorata* showed better repellency than *B. balsamifera* and *V. trifolia* in 30 min, although its MED value is higher than theirs. DEET provides much longer complete protection time than most plant extracts (Tawatsin et al., 2006; Wu et al., 2019), including *A. indica* oil which showed a lower MED. The reason for the rapid decline in the repellency of most plant extracts may be due to their low boiling points, which impact the remain time of the film on the skin.

A. indica is the most effective mosquito repellents, consistent with its extremely high popularity in folk. There is no previous repellent test of *A. indica* against mosquitoes. Compounds identified from its essential oils contain volatile constituents as mosquito repellent, including borneol, camphor, eucalyptol, and (+)-α-terpineol (Wu et al., 2008). *Artemisia vulgaris* of the same genus is also an insect-repelling plant traditionally used by people in many regions of China. It showed strong mosquito-repelling activity, and its essential oil contained multiple repellent compounds (Hwang et al., 1985). Screening highly effective compounds and/or combinations can be achieved through

Table 4
An overview of previous related ethnobotanical reports, mosquito repellent efficacy and major compounds from literature review of plant species mentioned by Dai villagers in Xishuangbanna.

Plant species	Target of repellent in previous ethnobotanical report (Study area)	Mosquito repellency			Major compounds of essential oils
		Extraction (parts) Repellency	Mosquito-dose-time-repellency (%)	Reference	
Ageratina adenophora	Mosquitoes (China); Fan et al. (2019)	Methanol Extract	*Aedes aegypti*-5mg/cm^2-120min-100% *Aedes aegypti*-2.5mg/cm^2-90min-100%	Rajeswary and Govindarajan, (2013)	Amorph-4-en-7-ol, Bornyl acetate, **p-Cymene**, 3-Acetoxyamorpha-4,7(11)-dien-8-one, α-Phellandrene, Camphene, **α-Bisabolol**, α-Cadinol. Padalia et al. (2009)
Ageratum conyzoides	Insects (Bangladesh, India); Choudhury et al. (2011); Rahmatullah et al. (2010) Mosquitoes (China); Huang and Long (2013)	Essential oils (Leaves) Essential oils (Flowers)	*Aedes aegypti*-3.33uL/cm^2-60min; 1.67uL/cm^2-39min *Aedes aegypti*-3.33uL/cm^2-40min; 1.67uL/cm^2-30min	Trongtokit et al. (2005) Trongtokit et al. (2005)	Precocene I, Precocene II, 3,3-Dimethyl-5-tert-butylindone, **β-Caryophyllene**, γ-Bisabolene, Fenchyl acetate. Kong et al. (1999)
Artemisia indica	Insects (Nepal); Koul et al. (2018)	–	–	–	Caryophyllene(12.96), **Borneol**(12.6), Germacrene D(9.99), **Camphor**(8.05), [1S-(1α,2β,4β)]-1-methyl-1-ethenyl-2,4-bis(1-methylethenyl)-Cyclohexane (4.56), α-Caryophyllene(3.42), 6,6-dimethyl-Bicyclo [3.1.1]hept-2-ene-2-methanol (2.42), (1S-cis)-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-Naphthalene (2.22), [1R-(1α,7β,8aα)]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-Naphthalene (2.04), **Eucalyptol (1.46)**, **(+)-α-Terpineol** (1.11) Wu et al. (2008)
Blumea balsamifera	Insects (Philippines); Obico and Ragragio (2014) Mites and bedbugs (Laos); De Boer et al. (2010) Mosquitoes (China); Huang and Long (2013)	–	–	–	**1,8-Cineole** (20.98), **Borneol** (11.99), **β-Caryophyllene** (10.38), **Camphor** (8.06), **4-Terpineol (6.49)**, **α-Terpineol** (5.91), **Caryophyllene oxide** (5.35), β-Thujone (3.21) Chu et al. (2012)
Chromolaena odorata	Insects (India, Philippines, Bangladesh); Choudhury et al. (2011); Mollik et al. (2010); Obico and Ragragio (2014) Insects from stored grains (Ghana); Cobbinah et al. (1999) Mosquitoes (Cameroon, China); Moore (2005); Youmsi et al. (2017)	Essential oils Methanol Extraction (Leaves)	*Aedes aegypti*-3.33uL/cm^2-30min *Anopheles arabiensis*-1mg-2min-80%	Trongtokit et al. (2005) Maharaj et al. (2010)	trans-Caryophyllene (16.58 %), δ-Cadinene (15.85 %), α-Copaene (11.58%), **Caryophyllene oxide** (9.63 %), Germacrene D (4.96 %), **α-Humulene** (4.32 %) Ling et al. (2003) **α-Pinene** (42.2%), **β-Pinene** (10.6%), Germacrene D (9.7%), β-Copaen-4α-ol (9.4%), (*E*)-Caryophyllene (5.4%), and Geijerene/Pregeijerene (7.5%) Owolabi et al. (2010)
Clausena excavata	Mosquitoes (South Africa, Laos); De Boer et al. (2010); Mavundza et al. (2011) Mosquitoes, fleas and chicken mites (China); Fan et al. (2019)	–	–	–	**β-Caryophyllene,** Elixene, Methoxyeugenol, β-Cubebene, δ-Cadinene, Spathulenol, γ-Muurolene, Aromadendrene, Globulol. Guo et al. (2018)
Clerodendrum bungei	Mosquitoes, chicken lices (China); Fan et al. (2019)	–	–	–	**Linalool (28.53)**, 1-Octen-3-ol (11.9), 1-Hexanol (10.43), 3-Oectanone (6.35), **Benzaldehyde (6.25).** Song et al. (2007)
Curcuma longa	Mosquitoes (China); Fan et al. (2019); Huang and Long (2013) Ticks (Laos); De Boer et al. (2010);	Essential oils	*Aedes aegypti*-3.33uL/cm^2-10min	Trongtokit et al. (2005)	Tumerone (41.11), ar-Tumerone (23.12), Curlone (19.14), α-Phellandrene (5.04), **Eucalyptol** (3.92), 1-Methyl-2-(1-methylethyl)-benzene (1.66), 2,2-Dicyclohexylmalononitrile (1.28). Tawatsin et al. (2006)
		Essential oils	*Aedes aegypti*-0.33uL/cm^2-2.7 h *Aedes albopictus*-0.33uL/cm^2-8.0 h *Anopheles dirus*-0.33uL/cm^2-8.0 h *Culex quinquefasciatus*-0.33uL/cm^2-8.0 h	Tawatsin et al. (2006)	
Datura metel	Mites (Laos); De Boer et al. (2010)	–	–	–	Ketone (18.84%), **Phytol** (18.71%). Xue et al. (2016)
Dysphania ambrosioides	Mosquitoes, louse, and chicken mites (China); Fan et al. (2019) Stored grain insects (Africa); Malik and Mujtaba Naqvi (1984)	Essential oils DCM/ Methanol (1:1) Extraction (Leaves)	*Aedes aegypti*-7.64uL/cm^2-60min-100% *Anopheles arabiensis*-2min-87%	Gillij et al. (2008) Maharaj et al. (2010)	(Z)-Ascaridole(29.7), Isoascaridole(13.0), **ρ-Cymene**(12.7), Piperitone(5.0), **Carvacrol**(4.9), 3,4-Epoxy-ρ-menthan-2-on(4.1), α,α-4-Trimethylbenzyl alcohol(2.8), **Caryophyllene oxide**(2.2), δ-4-Carene(1.9), Hexahydrofarnesyl acetone(1.7), Precocene II (1.5), **α-Pinene**(1.3), **Phytol**(1.2), **α-Terpinene**(1.1), **Thymol**(1.1) Chu (2011)
		–	–	–	

(*continued on next page*)

Table 4 (*continued*)

Plant species	Target of repellent in previous ethnobotanical report (Study area)	Mosquito repellency			Major compounds of essential oils
		Extraction (parts) Repellency	Mosquito-dose-time-repellency (%)	Reference	
Laggera pterodonta	Mosquitoes (Cameroon); Njan-Nloga et al. (2007)				Isoeugenol (34.06%), [S,(−)]-2,3,4,4a,5,6-Hexahydro-1, 4a-Dimethyl-7-(1-methylethyl) naphthalene (24.78%), α-Caryophyllene (8.19%), Eudesm-7(11)-en-4-ol (5.51%), Eudesma-3,7(11)-diene (4.43%), **β-caryophyllene** (3.70%). Guo (2017)
Lantana camara	Housefly (Uganda); Baana et al. (2018); Insects (Philippines, Yemen); Hussein and Dhabe (2018); Obico and Ragragio (2014) Mosquitoes (Tanzania, Kenya, India, China, Nigeria); Egunyomi et al. (2010); Huang and Long (2013); Kweka et al. (2008); Namsa et al. (2011); Seyoum et al. (2002)	Ethyl acetate Extraction (Leaves) Methanol extraction (Leaves)	*Anopheles stephensi*-500 ppm-30min-98% *Culex quinquefasciatus*-500 ppm-30min-93% *Anopheles stephensi*-500 ppm-30min-82% *Culex quinquefasciatus*-500 ppm-30min-86%	Kamaraj et al. (2011) Kamaraj et al. (2011)	Germacrene D (15.85%), **β-Caryophyllene** (12.35%), **α-Humulene** (9.31%), Germacrene-B (6.19%), Bicyclogermacrene (4.7), **1,8-Cineole** (4.61%). Sundufu and Huang (2004)
Litsea cubeba	Mosquitoes (China); Fan et al. (2019); Moore (2005) Mosquitoes and bedbugs (Laos); De Boer et al. (2010);	Essential oils Essential oils	*Aedes aegypti*-0.33uL/cm^2-1.8 h *Aedes albopictus*-0.33uL/cm^2-6.3 h *Anopheles dirus*-0.33uL/cm^2-8.0 h *Culex quinquefasciatus*-0.33uL/cm^2-7.2 h *Aedes aegypti*-no repellent	Tawatsin et al. (2006) Trongtokit et al. (2005)	**Geranial** (27.49%), Neral (23.57%), **d-Limonene** (18.82%), β-Thujene (3.34%), **β-Pinene** (2.85%), **α-Pinene** (2.57%), 6-Methyl-5-hepten-2-one (2.40%), **Linalool** (2.36%). Yang et al. (2014)
Melia azedarach	Lice and mites (Laos); De Boer et al. (2010) Mosquitoes (Ethiopia, South Africa); Giday (2018); Karunamoorthi et al. (2009); Kenea and Tekie (2015); Kidane et al. (2013); Mavundza et al. (2011)	DCM/ Methanol (1:1) Extraction (Leaves)	*Anopheles arabiensis*-2min-60%	Maharaj et al. (2010)	**Caryophyllene oxide** (31.08%), Bicyclogermacrene (15.43%), Caryophyllene (9.11%), Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-(s)-(8.72%), Cycloisolongifolene,8,9-dehydro-(6.78%) Lei et al. (2010)
Microtoena patchoulii	Lice, silverfishes, cockroaches, mites (India); Yumkham et al. (2014)	–	–	–	**Patchouli alcohol** (54.4%), 1-Octen-3-ol (21.4%),α-Guaiene (2.4%), α-**Pinene** (2.1%), δ-Guaiene (2.1%), β-Selinene(1.7%), β-**Caryophyllene**(1.4%), δ-2-Carene (1.3%),α-Patchoulene (1.3%), β-**Pinene** (1.2%) Senpuku et al. (2007)
Nicotiana tabacum	Insects (Laos, China); De Boer et al. (2010); Fan et al. (2019) Mosquitoes (Kenya, Ethiopia, China) Kariuki et al. (2016); Kenea and Tekie (2015); Moore (2005) Ticks (Pakistan, Kenya); Farooq et al. (2008); Wanzala et al. (2012)	–	–	–	**Nicotine**. Boonyaroj and Amornkaew (2017)
Toona sinensis	–	–	–	–	**β-Caryophyllene** (19.51%), Humulen-(v1) (16.04%), Himachala-2, 4-diene (5.71%), Seychellene (4.82%), Longifolene-(V4) - (4.42%), **Caryophyllene oxide** (4.03%), Aristolene (3.18%) Chen et al. (2013)
Vitex trifolia	Mosquitoes, mayflies and stored grain pests (India); Yumkham et al. (2014)	Essential oils	*Aedes aegypti*-0.33uL/cm^2-2.3 h *Aedes albopictus*-0.33uL/cm^2-8.0 h *Anopheles dirus*-0.33uL/cm^2-8.0 h *Culex quinquefasciatus*-0.33uL/cm^2-7.8 h	Tawatsin et al. (2006)	**Eucalyptol** (31.26), p-Menth-1-en-8-ol acetate (13.48), β-Phellandrene (9.99), Caryophyllene (7.58), **α-Pinene** (6.93), p-Menth-1-en-8-ol (7.33), p-Menth-1-en-4-ol (4.57), (−)-Spathulenol (3.31), **Caryophyllene oxide** (2.97) Tawatsin et al. (2006)

Bold: Compounds that have been identified to have mosquito repellent activity (Bettarini et al., 1993; Boonyaroj and Amornkaew, 2017; Cantrell et al., 2016; Gokulakrishnan et al., 2013; Hwang et al., 1985; Paluch et al., 2009; Tabacan et al., 2016; Traboulsi et al., 2005; Wang et al., 2016).

bioassay-guided investigation of *A. indica* oils (Tabacan et al., 2013; Ma et al., 2019). To extend the protection time and greater economic value of *A. indica*, efforts should be made to develop new formulations, fixative additives and the production of combined repellents (Nerio et al., 2010; Wu et al., 2019).

4. Conclusions

Dai villagers in Xishuangbanna have a rich and diverse knowledge of plant-based remedies against insects. The current study is the first report of traditional use of *Datura metel, Microtoena patchouli* and *Toona sinensis* as mosquito repellents. The methods for controlling mosquitoes were diverse: direct burning was used for most plants, followed by smearing,

Table 5
Minimum effective dosage (MED) per minute of 10 plant extracts that prevent mosquitoes from biting.

Plant	Extraction	MED (mg/cm^2)
Artemisia indica	Essential oil	0.015 ± 0.005
Ageratum conyzoides	Essential oil	0.250 ± 0.085
Blumea balsamifera	Essential oil	0.090 ± 0.000
Clausena excavata	Essential oil	0.250 ± 0.085
Clerodendrum bungei	Petroleum ether extract	0.370 ± 0.000
Ageratina adenophora	Essential oil	0.277 ± 0.132
Chromolaena odorata	Essential oil	0.109 ± 0.132
Laggera pterodonta。	Essential oil	0.433 ± 0.229
Nicotiana tabacum	Petroleum ether extract	0.061 ± 0.020
Vitex trifolia	Essential oil	0.090 ± 0.000
DEET		0.019 ± 0.006

Table 6
Time dependent mosquito repellent activity of different concentration of different test substances against *Ae. albopictus*.

Plant	Concentration (mg/cm^2)	Mean[a] repellency (±S.D.)/%			
		30 min	60 min	90 min	120 min
Artemisia indica	0.45	100.00 ± 0.00a	98.72 ± 2.57a	94.80 ± 6.00ab	92.15 ± 1.68a
	0.3	98.16 ± 3.68a	92.68 ± 6.80ab	91.15 ± 10.20ab	70.42 ± 22.09b
	0.15	90.80 ± 9.30ab	85.29 ± 14.10abc	81.44 ± 11.22bc	66.40 ± 16.62b
Blumea balsamifera	0.45	85.18 ± 8.44bc	65.47 ± 8.97de	54.79 ± 15.27def	–
	0.3	88.94 ± 5.25ab	80.60 ± 8.91bcd	77.20 ± 10.81bc	73.76 ± 2.56b
	0.15	66.73 ± 13.46d	53.27 ± 15.27e	41.00 ± 16.52f	–
Chromolaena odorata	0.45	91.73 ± 10.34ab	78.4 ± 9.00bcd	77.92 ± 12.50bc	57.86 ± 13.12b
	0.3	98.48 ± 1.79a	90.03 ± 6.77ab	65.79 ± 9.58cde	65.24 ± 11.91b
	0.15	83.58 ± 11.56bc	58.88 ± 15.36e	44.80 ± 7.20f	–
Nicotiana tabacum	0.45	64.62 ± 6.37d	56.06 ± 16.56e	52.45 ± 12.39def	–
	0.3	74.71 ± 5.01cd	59.89 ± 20.78e	38.46 ± 9.65f	–
	0.15	65.25 ± 6.25d	66.25 ± 7.49de	48.45 ± 16.12df	–
Vitex trifolia	0.45	81.54 ± 8.17bc	63.32 ± 15.64de	43.02 ± 13.50f	–
	0.3	83.97 ± 11.97bc	77.29 ± 17.16bcd	68.10 ± 13.61cd	–
	0.15	81.89 ± 12.90bc	69.46 ± 12.87cde	64.91 ± 7.45cde	–
DEET	0.45	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	0.3	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	0.15	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

[a] Different letters followed mean repellency percentages show significant difference ($p < 0.05$, by one-way ANOVA with Duncan's multiple range test) when the comparison of mosquito repellency was made between different test repellent (including different concentrations of five plants and DEET) after a specific time interval (30, 60, 90 and 120 min) independently.

and placing. Essential oils of all reported plants contain main components as mosquito repellent. These plants are distributed widely and the results of this study can provide residents of other areas with mosquito repellent remedies based on local plant resources. Laboratory analyses confirmed that the ten plants with the higher popularity repel mosquitoes. *Artemisia indica* is the most effective mosquito repellents, consistent with its extremely high popularity in folk. Considering that the chemical components of plants vary among different regions, there is a need for bioassay-guided investigation of effective compounds and/or combinations of *A. indica* oils from this region. In order to develop these plants into mature natural mosquito repellents, future research should also focus on safety, new formulations, fixative additives and the production of combined repellents.

Declaration of competing interest

The authors declare no conflict of interest.

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Glossary

DEET	*N, N*-diethyl-3-methylbenzamide
FC	frequency of citation
MED	minimum effective dosage

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Contribution of authors

YG and YW conceptualized the study. YG, ZL, RF, CG and LW conducted ethnobotanical surveys. CG, YG and ZL collected and extracted plant materials. YG, HS, JL, CZ, CG and ZL perform laboratory trials. ZL and CW analyzed data. Manuscript was wrote by YG and revised by ZL and YW. All the authors have read and approved the final manuscript.

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