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Thesis

Application of Geographic Information System (GIS) and Comparison of Active Substances in Medicinal Plants Used in Malaria Treatment Based on Folk Medicine Wisdom in Sa Kaeo Province

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Abstract

Introduction: Malaria is a tropical disease caused by Plasmodium parasites. The parasite spreads to people through the bite of infected female Anopheles mosquitoes. Malaria occurs mainly in poor, tropical, and subtropical areas of the world. The use of traditional medicine/traditional healers is prominent among populations in low-income countries with malaria infection. This study investigated the use of medicinal plants in the treatment of malaria by traditional healers. As well as studying the chemicals in medicinal plants used in treating malaria. To develop information on traditional medicinal plants used to treat and prevent malaria. ***Methodology:*** In-depth interviews were conducted along with observational studies of traditional healers. ***Results:*** The results showed that 13 medicinal plants have been used in the treatment of malaria by the folk healers in the study areas. The medicinal plants most common used by folk healers are neem (Azadirachta indica A.Juss.), quinine (Brucea javanica (L.) Merr.), wormwood (Tinospora crispa (L.)), and Eurycoma longifolia Jack. These main medicinal plants have the nourishing, therapeutic and preventive properties for malaria during a journey in the forest. They have been used as analgesic against all kinds of fever such as persistent fever and malaria. Each folk healer has been taught by their ancestors with at least 15 years of experience in healing and taking care of a large number of patients in their community. As these 2 provinces are adjacent to Cambodia, all folk healers can treat malaria even in severe stages such as cerebral malaria, malaria-induced splenic infarction and other similar fever conditions. ***Conclusion:*** The active substances or phytochemicals of 5 medicinal plants used in the treatment of malaria were characterized using crude ethanol extracts. Two primary phytochemicals were identified in Azadirachta indica A.Juss. and Ficus Racemosa Linn. which were terpenoids and phenolics, two phytochemicals were identified in Brucea javanica (L.) Merr. which were alkaloids and phenolics while three phytochemicals were identified in Tinospora crispa (L.) and Eurycoma longifolia Jack which were terpenoids, alkaloids and phenolics. For the total alkaloid content in 3 medicinal plants, Brucea javanica (L.) Merr. had highest total alkaloid content of 1.73-1.84%, followed by Tinospora crispa (L.) 1.15-1.59% and Eurycoma longifolia Jack 0.34-0.58%.

In conclusion, folk healers in Sa Kaeo Province used medicinal plants gathered from their local community forests, which are found in every district, according to a study on the distribution and density of medicinal plants used in the treatment and prevention of malaria in the province's various districts. These therapeutic plants' coordinates were found using the geographic information system (GIS).

Keywords: ***Geographic information system, Active substances of medicinal plants, Treatment of malaria, Folk healer wisdom.***

Introduction

In Thai traditional medicine for malaria treatment, it was originally believed that malaria was associated with swamps. Hippocrates, the Father of Medicine, said that drinking water in a swamp can lead to malaria, so malaria was initially called “swamp fever” (Phonrat Wilairat and Sornchai Luareesuwan, 2002). During the reign of King Rama IV, Krom Luang Wongsathirat, a famous Thai traditional medicine doctor in the treatment of diseases who had knowledge of both Thai traditional medicine and Western medicine used most of traditional Thai traditional medicine approaches and sometimes adopted Western medicine in combination with Thai medicine formulations. For example, use of quinine for fever by wrapping it inside a bolus (Journal of Thai Traditional and Alternative Medicine, 2008). In one of his pharmacopoeias, 42 formulations of Western medicines are mentioned and compiled into categories and it is the first materia medica pharmacopoeia in Thailand that mentioned Western medicines (Prachot Plengwittaya, 1983, 139 -140). One of Western medicines he mentioned is Singkona, extracted from *Syngonium macrophyllum* bark, which is used as a malaria remedy. There are currently several regimens for drug-resistant malaria, especially the combination of medicines into new formulations used

for different types of malaria. Malaria is still a major problem in the world and Thailand, especially drug resistance. Two plants are known to have excellent malaria therapeutic properties: Cinchona (its bark is used to produce quinine) and Qinghaosu plants. Later, Chinese scientists developed a Chinese herbal recipe called "Kot Chulalumpa" which was found to be highly effective against malaria parasites.

Even though Thailand is making great strides in treating malaria, the cost of importing medications from abroad is still high. A budget plan of 214,167,499 Baht was planned in 2020 for the Thailand Malaria Elimination Project; however, as of 2017, the actual expenses incurred amount to 31,649,258 baht (Insect-Borne Disease Division, 2017). Extraction of active substances from domestic herbs and medicine manufacturing for domestic consumption may lower import costs and may also lead to the export of Thai herb-derived medications or active ingredients to other nations, generating revenue for the Thai people. It is important to determine and characterize the active ingredients for malaria inhibition is to study the content of active substances in various medicinal plants. This is in order to compare and select medicinal plants to be used as raw materials in pharmaceutical production. In addition, it is important to establish a geographic information system for herbs as an important resource for future research and, most importantly, to have broader implications for local farmers, cultivation and propagation of those essential herbs at industrial-scale. It is also beneficial to the security of public health, reducing the cost of importing drugs from abroad, or may be used for distribution in order to generate income in the future.

Therefore, the researcher was interested in studying the use of medicinal plants in malaria treatment by folk healers by determining the active substances of medicinal plant samples used in malaria treatment in each area. This is in order to use and apply the geographic information and data obtained to alternative medical treatment and for the benefits of public health community and patients.

Objectives

1. To study the use of medicinal plants used in malaria treatment by folk healers
2. To determine the active substances of the main medicinal plants used in malaria treatment
3. To develop information on local herbs used in malaria treatment and prevention.

Research Questions

1. What are the uses of medicinal plants in malaria treatment by folk healers?
2. What are the active substances of the medicinal plants used in malaria treatment?
3. How is information developed on traditional herbs used in the treatment and prevention of malaria?

Literature Review

Application of geographic information system (GIS) and comparison of active substances in medicinal plants used in malaria treatment based on folk medicine wisdom in Sa Kaeo Province is based on the following knowledge, concepts, theories, and researches.

1. *Knowledge on application of geographic information systems (GIS) in public health:* Review of applications of GIS in 4 areas of public health: communicable disease control, non-communicable disease management, health promotion, public health planning and environment, safety, and occupational health. The use of GIS in epidemiological studies to depict disease scenarios or the epidemiologic triad, as well as the display of spatial data to identify disease areas, temporal distribution, locations, and population groups, are a few examples of GIS applications in the control of communicable diseases (Tipaya Mongkholgul, 2012). This enhances public health care and increases the effectiveness of planning for the prevention and control of communicable diseases (Kongmung, 2010).
2. Identification of active substances in medicinal plants used in Thai traditional medicine involves several aspects, one of which is the improvement of accuracy verification for global recognition. As a result, attempts have been made to identify and document the active substances in medicinal plants. There are approximately 11 common active substance in medicinal plants which are alkaloids, anthraquinones, terpenoids, saponins, tannins, flavonoid, volatile oil, resin, oleoresin, gum resin, and balsam. Herbs' pharmacological effects, therapeutic qualities, and flavor are all influenced by their active substances.
3. *Treatment for Malaria:* In addition to AIDS, dengue fever, and tuberculosis, the World Health Organization (WHO) has identified malaria as one of the four illnesses that urgently need medical attention. The term "Malaria" is originated from the Italian term for bad air because it caused by Anopheles mosquitoes that transmit this disease to humans and Tropical regions with copious amounts of precipitation and naturally occurring standing water are home to Anopheles mosquitoes. Such weather condition is thought to have contributed to up to 216 million cases of malaria infection in 2010 and around 6,550,000 fatalities (81% of all cases) in Africa. Malaria is a fatal disease that kills Thai people who live in the mountainous forests of Thailand. Numerous terms are used to refer to malaria, including teak blossom fever, Pang fever, jungle fever, and hot and cold fever.

In conclusion, despite a decline in infections, malaria is still a serious issue in many parts of the world where new outbreaks are being recorded. This is caused by a number of factors, such as movement of malaria patients and mosquito (malaria vectors), as well as social, economic, and environmental factors that make it difficult to implement ongoing controls to stop the spread of this disease. Additionally, antibiotic resistance is a significant issue that reduces the effectiveness of treatment for infected people. Drug-resistant strains are currently spreading around the world and becoming increasingly prevalent. Thus, in order to halt violence and its effects on humanity both now and in the future, it is necessary to develop and characterize medicinal properties of medicinal plants, which requires cooperation from all interested parties.

4. Related concepts and theories

4.1 Concept of medical anthropology

The concept of medical anthropology emphasizes the value of folk medicine, which uses the social context to treat patients successfully and finally integrates into the social structure and culture. In this study, malaria treatment in public health, modern medicine and folk medicine were analyzed using anthropology concept and theory.

4.2 Structural Functional Theory

One of the six components of social structure, according to the structural functional theory, is health, which can be connected to other components to create a secure and stable society. In this study, this principle was applied in an analysis of treatment model for malaria patients.

4.3 Ecological theory

According to ecological theory, humans should live according to their own ecological systems, which provide them with clothing, food, shelter, and other necessities that varies depending on where they are in the globe. Cultural ecology is the science of the relationship between the environment and human way of life. According to this theory, people from different cultures relate to or adapt to their surroundings. Using this principle as a guideline, the researcher has examined habitats and medicinal plants used to treat malaria patients, particularly in the eastern region, Cambodia–Thailand border.

4.4 Diffusion theory

Diffusion theory is a theory that explains health culture. According to this theory, in order to address their basic needs, people develop culture wherever they go, and that this culture has an origin and spreads across the areas. Cultural diffusion from one community to another is the reason why many societies have the same culture. Societies can share a culture without necessarily sharing a common ancestor when cultural acceptance is present. The dissemination and exchange between treatment approaches was examined in this study by analyzing treatment methods for patients with malaria using the theoretical framework.

5. Related researches

5.1 Researches in Thailand

We recognize that extracts from several medicinal plants can prevent malaria, including the transmission and issues encountered in the transmission of malaria in Thailand, based on local wisdom that suggests many local medicinal plants have antimalarial properties. These data served as the foundational knowledge for this study.

5.2 Researches in other countries

Research has been conducted on extraction of active substances from local medicinal plants and their antimalarial properties. Scientific experiments have shown that active substances in many plants are as effective as antimalarial medications. Furthermore, in order to develop a malaria control planning model, geographic information systems are currently being employed as a database for local malaria control. The development of this system makes it easier to identify high-risk and case-count areas for malaria. These data served as the foundational knowledge for this study.

Conceptual Framework

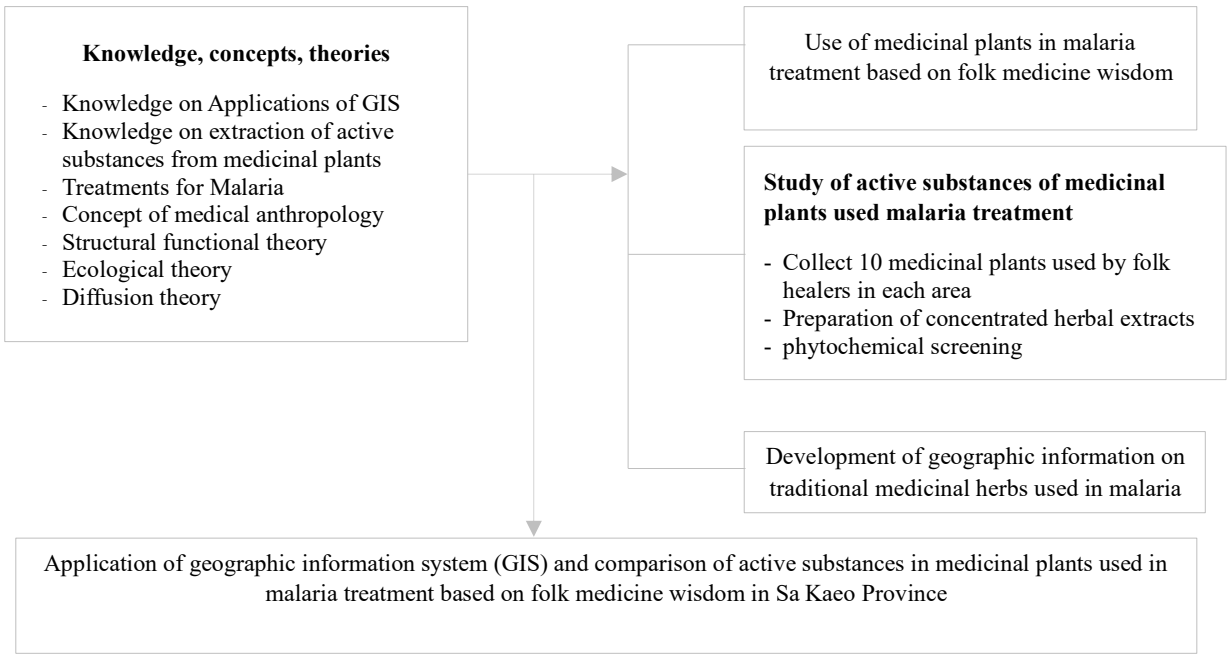

Figure 1 Conceptual Framework

Methodology

The objectives of this study were to study the use of medicinal plants used in malaria treatment by folk healers and to develop information on local herbs used in malaria treatment. This study was applied research using mixed methods. The quantitative research methodology was used to create a geographic information system (GIS). Qualitative research methodology was also used in the data collection from related documents, field study, observation, interview, and focus group discussion. The data obtained were then analyzed (Songkoon Chantachon, 2020: 118-151). This research procedure was approved by the Human Research Ethics Committee, Mahasarakham University, with the registration number 35/2020

Population and Sample

The population in this study were people who involved in malaria treatment using herbs which were selected using purposive sampling and the sample consisted of: Two folk healers who specialize in the use of medicinal plants in malaria treatment in Sa Kaeo Province. Two officials with knowledge and expertise in information and surveillance of vector borne diseases from the Bureau of Vector Borne Diseases, Department of Disease Control, Sa Kaeo Province. Two officials with expertise in vector borne diseases treatment in Sa Kaeo Province.

Instrument and Material The research instrument used in 3 phases of this study according to the objectives are as follows.

1. In the study of the use of medicinal plants in malaria treatment by folk healers the researcher collected primary data using a structured interview, an in-depth interview, and observation which included participant observation and non-participant observation, as well as focus group discussion, note-taking equipment, voice recorder and camera.
2. The instrument used in comparison of active substances in medicinal plants used in malaria treatment included field notes, a scale with 2 decimal places, and a rotary

evaporator, water bath, 500 ml beaker, 500 ml Erlenmeyer flask, and chemicals used included 95% ethanol, conc. HCl, iodine, potassium iodide, magnesium wire, 10% H_2SO_4, 10% NH_3, 1% $FeCl_3$, dichloromethane and glacial acetic acid.

3. The development of information on local medicinal herbs used in malaria treatment and prevention involves survey of main medicinal plants in the study area aiming to develop a geographic information system (GIS). In this process, spatial data was processed using a computer system in order to identify spatial location of each herb, create a geographic information system using vector map, global positioning system device (GPS), GIS software package, camera and sound recorder.

Research Operation

1. In order to collect the necessary data for the study on the use of medicinal plants in malaria treatment by folk healers, the researcher conducted both structured and in-depth interviews. This is because an interview allows for flexibility. It gives the interviewer the chance to clarify the purpose of each question to the interviewee so that they understand it better, as well as the chance to ask additional questions and offer comprehensive details on a range of issues. During an interview, mutual understanding between the interviewer and the interviewee can be verified. Moreover, the interviewee has the ability to organize and present the experiences in their own perspective, and the interviewee's behaviors may be seen throughout the interview. In this study, an interview guideline was developed based on the research objectives and problems and the basic research framework. In addition, focus group discussion was also used in the data collection for the purpose of data verification.
2. The active substances of the main medicinal plant samples used in malaria treatment were identified through classification of active substances of each medicinal plant in the study area and collection of medicinal plant samples. In this study, 10 medicinal plants used by folk healers in each area were collected. Their species were verified by the botanists and the identification of actives substances were conducted and compared in the laboratory. The research instrument used were identification of medicinal plants by botanists, field notes, and scientific laboratories.

   In preparation of herbal extracts, herbs were cut into small pieces, dried, and weighed to 100 g on a scale with two decimal places. After the herb sample ground into fine powder, it was mixed with 200 ml of 95% ethanol in a 500 ml beaker. The mixture was left in the beaker for 4 days after it was covered with aluminum foil. The mixture was stirred daily, filtered and stored it in a 250 ml Erlenmeyer flask covered with aluminum foil. In a beaker, the residue was mixed with 200 ml of 95% ethanol. After covering the beaker with aluminum foil, the mixture was left for another 4 days. These extraction steps were repeated 4 times. The solvent in the pooled mixture was evaporate using a rotary evaporator in order to obtain concentrated herbal extracts.

   Phytochemical screening involved identification of chemical compounds present in plants using colorimetric reactions or precipitation. It categorized secondary metabolites into seven groups: alkaloids, flavonoids, anthraquinones, saponins, tannins, terpenoids, and cardiac glycosides (Ayoola et al., 2013) as follows.

a) **Alkaloids:** Wagner's Reagent was prepared by dissolving 2 g of iodine and 6 g of potassium iodide in 100 ml of water. 1.0 ml of 1.5% v/v HCl was added to 0.2 g of extract. The mixture was then mixed and place in a water bath for 5 min. After filtering off the insoluble fraction, the filtrate was let to cool to room temperature. Five drops of Wagner's Reagent were dropped in the filtrate. Yellow precipitate formation after shaking indicated the existence of alkaloids.

b) **Flavonoids:** 1.0 ml of 50% ethanol was added to 0.2 g of extract. The mixture was shaken and the insolutble fraction was filtered out. A piece of magnesium wire was added in the filtrate and 5 drops of conc. HCl were then added to the filtrate. The color change of the filtrate to dark yellow indicated the presence of flavonoids.

c) **Anthraquinones:** 0.2 g of extract was added to 10 ml of 10% H_2SO_4. The mixture was shaken and placed in a water bath for 5 min. After filtering off the insoluble fraction, the filtrate was let to cool to room temperature. 0.5 ml of 10% NH_3 was then added to the filtrate. The color change of the filtrate to pink-red indicated the presence of anthraquinones.

d) **Saponins:** The presence of saponins was verified through bubble formation. 0.2 g of extract was added to 5.0 ml of distilled water. The mixture was then placed in a water bath for 5 min. The bubble formation after shaking vigorously indicated the presence of saponins.

e) **Tannins:** 0.2 g of extract was added to 1.0 ml of distilled water. The mixture was then placed in a water bath for 5 min. After filtering off the insoluble fraction, 5 drops of 1% $FeCl_3$ were added to the filtrate. The color change of the filtrate to dark green or dark blue indicated the presence of tannins.

f) **Terpenoids:** 0.2 g of extract was added to 1.0 mL of dichloromethane. The mixture was then shaken. After filtering off the insoluble fraction, 0.5 mL conc. H_2SO_3 was slowly added to the filtrate. The formation of a brown ring at the interface of extract layer and sulfuric acid indicated the presence of terpenoids.

g) **Cardiac glycosides:** 0.2 g of extract was dissolved in 1.0 ml of dichloromethane by shaking. After filtering off the insoluble fraction, 5 drops of 1% $FeCl_3$ were added to the filtrate. The mixture was shaken and 5 drops of glacial acetic acid was then added to the mixture. After that, 0.5 ml of conc. H_2SO_4 was slowly added to the mixture. The formation of a brown ring at the interface of extract and sulfuric acid indicated the presence of cardiac glycosides.

3. The development of information on local medicinal herbs used in malaria treatment and prevention involved classification of coordinates and locations of medicinal plants in the study area. The photos of medicinal plants found in the study area were taken and their coordinated were determined using a GPS device. A GIS software package was then used in data processing and development of GIS database.

Data Analysis

1. The use of medicinal plants in malaria treatment by folk healers was studied concurrently with preliminary data verification throughout the study period. Upon completion of field data collection, the data obtained from recordings, interview transcripts, and photography were categorized. After that, triangulation was used to confirm the data credibility and completeness and make sure it satisfied the research goals. If any part of the information is found to be incomplete, additional information will be collected in that part.
2. Comparison of active substances in each medicinal plant used in malaria treatment was conducted.

3. The development of information on local medicinal herbs used in malaria treatment and prevention involved determination of coordinates and locations of medicinal plants using the data obtained and a GPS device in order to develop a GIS database.

Overview of research findings were presented using descriptive analysis.

Results

1. Use of medicinal plants in malaria treatment by folk healers

The results showed that 13 medicinal plants have been used by folk healers in the treatment of malaria by the folk healers in the study area. The medicinal plants most common used by folk healers are Neem (*Azadirachta indica* A.Juss.), Quinine (*Brucea javanica* (L.) Merr.), Wormwood (*Tinospora crispa* (L.)), and *Eurycoma longifolia* Jack. According to the in-depth interview with folk healers, these main medicinal plants have the nourishing, therapeutic and preventive properties for malaria during a journey in the forest. They have been used as analgesic against all kinds of fever such as persistent fever and malaria. Each folk healer has been taught by their ancestors with at least 15 years of experience in healing and taking care of a large number of patients in their community. As these 2 provinces are adjacent to Cambodia, folk healers believe that malaria infection in this area is more likely and more serious than in other areas of Thailand because the disease comes from border contact. All folk healers said that they can treat malaria even in severe stages such as cerebral malaria, malaria-induced splenic infarction and other similar fever conditions.

Use of medicinal plants in malaria treatment by folk healers

Folk wisdom of using medicinal plants in malaria treatment

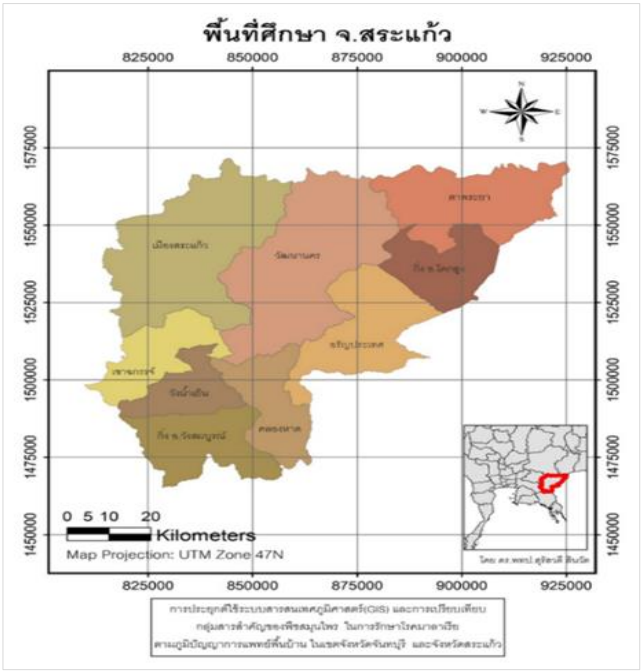

Figure 1: Study area for wisdom of malaria treatment of folk healers in Sa Kaeo Province

Sa Kaeo is a province in Eastern Thailand, and is a province under the Eastern Herbarium Development Project, located at latitude 13°45'0"N, longitude 102°19'60"E. From the past to the present, Sa Kaeo is considered one of the malaria epidemic areas. This is because most of the area is forest with numerous mosquito breeding grounds. In addition, it is adjacent to Cambodia, a country with severe malaria epidemic, and the people of the two countries can easily reach each other by land and sea routes for trading, business and doing orchard works. Due to the severity of the disease and inconvenient transportation in the past, when people in the community were sick, they were usually treated by folk medicine using local herbs. This is because the state's medical system was inaccessible to the community. Thai traditional medicine and folk healers therefore play an important role in treating patients in the community. Each folk healer has developed their treatment knowledge passed down from their ancestors and exchange of knowledge from other folk healers. In Sa Kaeo Province, there are several folk healers with experience and expertise in malaria treatment such as Mr. Saiyon Yaikratok and Mr. Lek Khemwilai.

1. Mr. Saiyon Yaikratok: He was born in Nakhon Ratchasima Province. He has 9 siblings and he is the second child. He moved to Sa Kaeo in 1979 when he was 23 years old. He currently resides at House No. 89, Village No. 15, Thung Maha Charoen Sub-district, Wang Nam Yen District, Sa Kaeo Province. He began studying herbs when he was about 6 or 7 years old because he had to collect herbs for his mother, who was a midwife. When he first came to Sa Kaeo, he became a midwife for the community. Malaria is caused by mosquito bites. Symptoms of malaria are shivering and chills, high fever, fatigue, lack of energy and white tongue. For example, one patient was from Cambodia. He was infected with malaria for 2-3 months and was not treated and eventually died. Malaria often spreads along the Dong Phaya Yen mountain range (formerly known as Dong Phaya Fai).

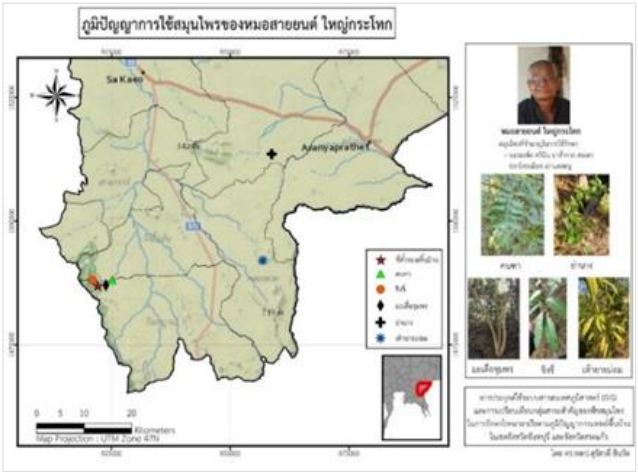

Figure 2: Five medicinal plants used in malaria treatment by Mr. Saiyon Yaikratok

Five-Root Recipe is mainly used in malaria treatment by Mr. Saiyon Yaikratok. Roots of five herbs are ground or crushed and mixed with boiled water to drink. Other herbs used in this recipe include jimsonweed (*Datura metel* L.) seeds, *Anamirta cocculus* L. vines, spider webs, wormwood (*Tinospora crispa* (L.)), neem (*Azadirachta indica* A.Juss.), eaglewood (*Aquilaria crassna* Pierre ex Lec.) and *Eurycoma longifolia* Jack roots. These herbs have a bitter taste. In the past, they were finely ground and mixed with half a cup of water to drink two times a day in the morning and evening. If it has a very bitter taste, Mr. Saiyon Yaikratok usually makes the patient takes about 2 tablespoons every 2 - 3 hours to release as much sweat as possible. If modern medicine is taken together with this remedy, the symptoms will improve in about 1 week. If only traditional medicine is used, symptoms will take approximately 7-10 days to recover and improve.

If there is a brain infection, the patient is treated with jimsonweed (*Datura metel* L.) seeds. Jimsonweed seeds are ground and mixed with white wine or lime juice or water from washing rice. **Instruction:** If the patient's weight is approximately 60 kilograms, one seed or 1 tablespoon of ground seed is used. If the patient's weight is 40-50 kilograms, half a seed is used. If the symptoms are severe and the patient has seizures, a handful of spider webs without

the fiber is also used in the decoction. When the patient takes this decoction, they will be calmer.

Figure 3: Mr. Saiyon Yaikratok explains the use of herbs in malaria treatment.

Figure 4: Traditional Thai medicine note by Mr. Saiyon Yaikratok

Figure 5: Spider webs used in preparation of lye

Spider web lye is prepared by mixing spider webs with water. The patient should drink about a cup of this liquid and then sleep. After the patient wakes up, have him drink this lye again. Spider web lye is one of the ingredients of the decoction. This lye is used when the folk healer considers that the patient has severe symptoms such as chills and twitching as if pulling a spider's web. After drinking spider web lye, the patient will be calmer. The Five-Pollens Recipe with heart nourishing effect is also used. This recipe consists of with Kob Cha Nang (*Pouzolzia pentandra* Benn.) Jan Khao (*Dracaena loureiroi* Gagnep.) and Kai Prakam (*Drypetes roxburghii* Wall.), Sura Marit (*Cocculus Laurifolius* DC.) and Ruesri Pasomset (*Plectranthus scutellarioides*).

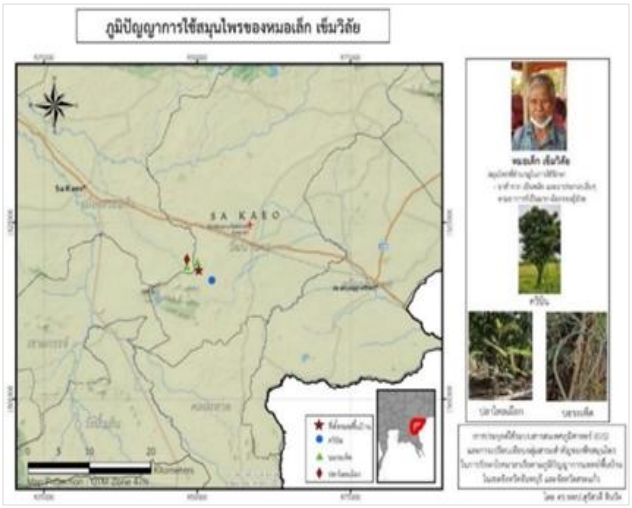

Figure 6: Map showing the locations of medicinal plants used in malaria treatment by Mr. Lek Khemwilai, folk healer in Sa Kaeo Province

2. Mr. Lek Khemwilai: He currently resides at House No. 69, Village No. 7, Huai Chot Sub-district, Watthana Nakhon District, Sa Kaeo Province. He has 3 siblings and he is the youngest. His original occupation was farmer. When his father died of diarrhea (cholera), he moved with his mother to Sa Kaeo Province and started growing cassava and corn, but the income was not good. Later, he turned his attention to studying herbs to help people in the community. He doesn't drink or smoke. He began studying at the primary level with Teacher Buntan Srichantha at Prachantakhan School. He learned herbal medicine from his father's textbooks. He has expertise in treating malaria.

Figure 7: Mr. Lek Khemwilai, a folk healer in Sa Kaeo, uses wormwood growing next to his house in malaria treatment

The decoction is prepared by boiling 300 grams of wormwood (fresh) in water until water decreases from 3 bowls to 1 bowl. This

decoction shall be taken before meals, 1 tablespoon at a time, 3 times a day (for about 7 days). Other herbs he used are such as quinine, Five-Root Recipe (recipe with roots of five herbs), *Eurycoma longifolia* Jack roots, wormwood, neem and *Mukia maderaspatana* Roem. tube. All these 5 herbs are washed thoroughly and boiled in water to drink for 7 days.

Figure *8: Eurycoma longifolia* Jack roots

Part 2: Active substances in medicinal plants used in malaria treatment

The phytochemical screening involves the following steps:

According to field data collection through in-depth interviews, folk healers in Chanthaburi and Sa Kaeo use different kinds of herbs in malaria patient treatment. However, the main herbs commonly used by these folk healers are neem, *Eurycoma longifolia* Jack. Root, wormwood vine, quinine bark and *Ficus Racemosa* Linn. A systematic literature review on chemical composition of all 5 medicinal plants was conducted. The results were as follows. Two primary phytochemicals are identified in *Azadirachta indica* A. Juss. and *Ficus racemosa* Linn. which are terpenoids and phenolics, two phytochemicals are identified in *Brucea javanica* (L.) Merr. which are alkaloids and phenolics while three phytochemicals are identified in *Tinospora crispa* (L.) and *Eurycoma longifolia* Jack which are terpenoids, alkaloids and phenolics.

Determination of total alkaloid content

For the total alkaloid content in 3 medicinal plants, *Brucea javanica* (L.) Merr. had highest total alkaloid content of 1.73-1.84%, followed by *Tinospora crispa* (L.) 1.15-1.59% and *Eurycoma longifolia* Jack 0.34-0.58%.

Determination of alkaloid content

Table 1: Quantity of herbal pellet obtained from extraction

Sample	Plant weight (g)	Volume of 20% acetic acid in ethanol (ml)	Pellet weight (g)	$\%\text{Yield} = \frac{\text{Substance weight} \times 100}{\text{Herb weight}}$
1. *Eurycoma longifolia* Jack., Sa Kaeo	40.0003	200	0.1389	0.34 %
2. Wormwood *(Tinospora crispa* (L.)), Sa Kaeo	40.0008	200	0.6353	1.59 %
3. Quinine (*Brucea javanica* (L.) Merr.), Sa Kaeo	40.0008	200	0.7372	1.84 %

Determination of total triterpenoid content

The determination of the total triterpenoid content in 4 medicinal plants revealed that dried powder of *Tinospora crispa* (L.) collected from Chantaburi had highest total triterpenoid content of 290.05 mg/g while the lowest total triterpenoid content of 6.35 mg/g were observed in dried powder of *Azadirachta indica* A.Juss. collected from Chantaburi.

Part 3: Development of geographic information system for local herbs used in malaria treatment

The data collected from documents, interviews, and focus group discussions were analyzed and used to identify the coordinates of herbs in the geographic information system for the hillside and mountainside areas.

Research tools include 1) global positioning system (GPS) device which is a system for finding positions on the earth; 2) satellite maps; 3) 1: 50,000 scale topographic maps; 4) compass; 5) 50-meter tape measure; 6) data recording devices; 7) sound recorder; 8) camera and 9) names of herbs. Field surveys were conducted by a team consisting of academics, local healers, and community leaders and various tools and equipment are used in both the rainy and dry seasons.

Mr. Saiyon Yaikratok, a folk healer in Wang Nam Yen District, has treated various diseases including malaria by using medicinal plants collected from their local community forest and more than 16 nearby places, with an area of approximately 718-3-20 rai. Herbs are also collected from the mountain forests of Sa Kaeo Province and herbs from other communities are traded. Wang Nam Yen District has a total area of 325.05 square kilometers (125.50 square miles) and a total population of 61,245 people (2021) with a density of 188.42 people/square kilometer. Given the high population density in Wang Nam Yen District, folk healers are faced with a large patient load. The researcher conducted a survey of the community forest as part of the process of creating a geographic information system. The community's ideas were utilized to gather, analyze, and present data that the researcher found valuable.

Mr. Lek Khemwilai, a folk healer in Watthana Nakhon District, has collected many important herbs in 25 community forests, which have a total area of 5,724-1-03 rai. This district has the largest number of community forests and the second largest area after Khok Sung District. Mr. Lek's village takes good care of its communal forests; extra herbs are planted, and a water system is made to keep the herbs hydrated. Watthana Nakhon District has a total area of 1,560.122 square kilometers (602.366 square miles) and a total population of 82,271 people (2021) with a density of 136.6 people/square kilometer. Given the high population density in Watthana Nakhon District, folk healers are faced with a large patient load. The results are as follows.

Distribution of medicinal plants in Sa Kaeo

There are a total of 83 community forests in Sa Kaeo, which are small but important forests with an area of 14,981 rai. Community forests are therefore a source of abundance with herbs, whether perennials, medium-sized trees, shrubs, vines, herbaceous plants, as well as tuber herbs. Folk healers has used herbs obtained from their community forests and other communities in different districts in malaria treatment. Herbs from other nature conservation forests are also used. More than 20 herbs have been used in malaria treatment. However, the main herbs commonly used by these folk healers are neem, *Eurycoma longifolia* Jack. Root, wormwood vine, quinine bark and *Ficus Racemosa* Linn. These herbs can be found mostly in community forests in Watthana Nakhon District, Khok Sung District, Wang Nam Yen District, Khao Chakan District, Mueang Sa Kaeo District, and Ta Phraya District.

Figure 9: Mr. Lek Khemwilai, a folk healer in Watthana Nakhon District, took the researcher to explore the herbal forest for creation of a geographic information system

4. Natural and water resources: Forests in Sa Kaeo Province include evergreen forests, mixed deciduous forests, and deciduous dipterocarp forests. Most of the forests in Sa Kaeo Province are characterized by mixed deciduous forest alternating with deciduous dipterocarp forest. According to the Royal Forest Department statistics 2016, Sa Kaeo Province has a forest area of approximately 923,796.63 rai, accounting for 20.5% of the province's area.

Community forests: Sa Kaeo has a large number of community forests in every district. Communities value their community forests because they provide for their four fundamental needs. In addition, these forests are home to valuable medicinal plants that can be used in patient treatment by folk healers. As a result, communal forests resemble the local supermarket. Community forests are long-standing natural forests and part of the community. They provide the community with a variety of plants beneficial for people in the community. As a result, the community treats its community forests with respect and makes use of them as needed.

Table 2: Community forests in Sa Kaeo Province (Source: Information Center, Royal

No.	District	Number (location)	Approximate area (rai)
1.	Mueang Sa Kaeo	13	534 - 3 – 68
2.	Watthana Nakhon	25	5,274 - 1 - 03
3.	Khao Chakan	3	280 - 3 – 12
4.	Wang Nam Yen	16	718 - 3 – 20
5.	Khlong Hat	3	302 - 0 – 04
6.	Aranyaprathet	5	47 - 0 – 00
7.	Ta Phraya	8	1,069 - 3 - 72
8.	Khok Sung	7	6,667 - 0 - 71
9.	Wang Sombun	3	87 - 0 - 00
Total		**83**	**14,981 - 3 - 50**

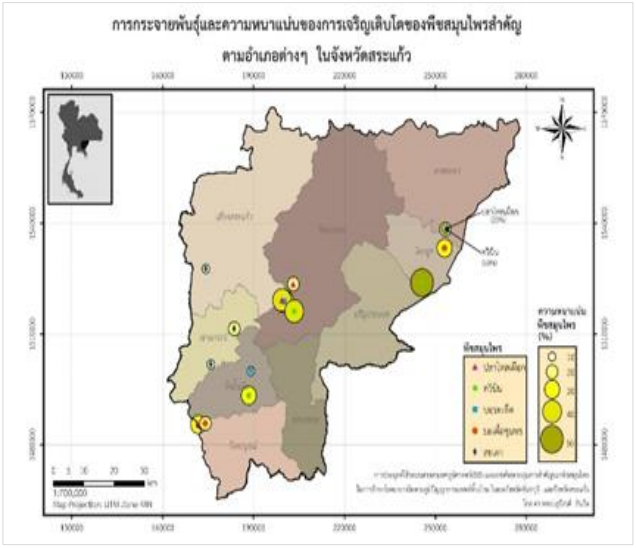

Figure 10: Distribution and density of medicinal plants found in different districts in Sa Kaeo

In this study, a survey was conducted and the data was used to create a geographic information system. According to the distribution and density of medicinal plants found in different districts in Sa Kaeo, Quinine (*Brucea javanica* (L.) Merr.) are widely distributed in Khok Sung District, Wang Nam Yen District and Ta Phraya District. *Eurycoma longifolia* Jack. are mostly found in Watthana Nakhon District, followed by Wang Nam Yen District. Neem (*Azadirachta indica* A.Juss.) are commonly found in Khao Chakan District. Wormwood (*Tinospora crispa* (L.)) are commonly found in Watthana Nakhon District. *Ficus Racemosa* Linn. are commonly found Wang Nam Yen District. In this community forest, there is a sprinkler irrigation system for medicinal plants, which shows that the community recognizes the importance of these medicinal plants.

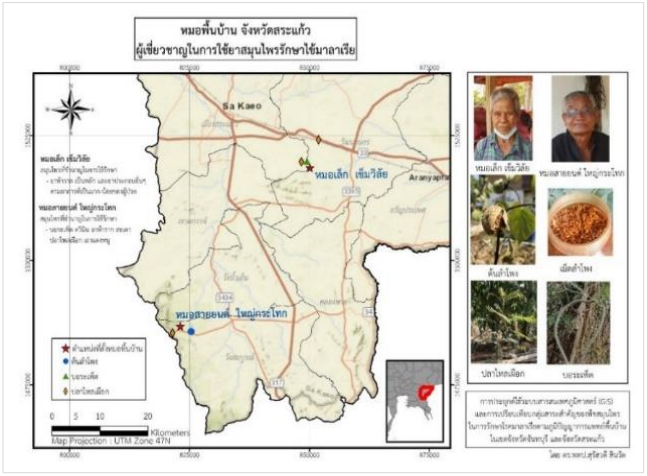

Figure 11: Geographic information of *Eurycoma longifolia* Jack. and Neem (*Azadirachta indica* A.Juss.), herbs commonly used by these folk healers in Sa Kaeo Province

Discussion

The discussion of the results is as follows.

1. Use of medicinal plants in malaria treatment by folk healers

1.1 The results showed that because of the hilly terrain and abundance of medicinal plants, people in Chanthaburi and Sa Kaeo provinces have used medicinal plants in their daily lives. Medicinal plants are a part of local lifestyle due to it availability, especially for treating illnesses. The use of such medicinal plants has been passed down from generation to generation and has become knowledge or folk wisdom. This knowledge comes from trial and error and experience accumulated over a long period of time. The results showed that 13 medicinal plants have been used in malaria treatment by folk healers in the study area. The medicinal plants most common used by folk healers are Neem (*Azadirachta indica* A.Juss.), Quinine

(*Brucea javanica* (L.) Merr.), Wormwood (*Tinospora crispa* (L.)), and *Eurycoma longifolia* Jack. According to the in-depth interview with folk healers, these main medicinal plants have the nourishing, therapeutic and preventive properties for malaria during a journey in the forest. They have been used as analgesic against all kinds of fever such as persistent fever and malaria. Each folk healer has been taught by their ancestors with at least 15 years of experience in healing and taking care of a large number of patients in their community.

The related, important theory is Ecology Theory, which describes the interactions between humans and the environment. Julian Steward and others such as Andrew P. Williams and Roy A. Rappaport (1968), ecologists, were interested in nature as a determinant of human behavior and have extended on this idea that the environment has a strong connection with human nature. The ecological theory highlights how human survival is reliant on ecological systems, whether food, shelter, medicine, and clothing, all of which vary depending on where you live. This theory was used in an analysis of habitats and medicinal plants used in malaria treat treatment, especially in the eastern region where the border between Thailand and Cambodia is located. Ecology is the scientific study of the interaction between human existence and the environment. According to this theory, people across cultures have a relationship with their environment or adapt to them (Songkoon Chantachon. 2020: 54-55).

2. Active substances in medicinal plants used in malaria treatment

2.1 The active substances or phytochemicals of 5 medicinal plants used in the treatment of malaria were characterized using crude ethanol extracts. Two primary phytochemicals were identified in *Azadirachta indica* A.Juss. and *Ficus racemosa* Linn. which were terpenoids and phenolics, two phytochemicals were identified in *Brucea javanica* (L.) Merr. which were alkaloids and phenolics while three phytochemicals were identified in *Tinospora crispa* (L.) and *Eurycoma longifolia* Jack which were terpenoids, alkaloids and phenolics.

According to the above findings, phenolics are phytochemicals found in all 5 medicinal plants, namely neem leaves, *Eurycoma longifolia* Jack roots, wormwood vines, quinine leaves, and *Ficus Racemosa* Linn. roots.

This is in line with the findings from a study on determination of anti-herpes virus and anti-malaria active substances of local plants in Thailand aiming to find the best extraction method for 17 active substances of local plants in order to investigate their biological activity against malaria using 95% ethanol as a solvent.

3. Development of geographic information system for local herbs used in malaria treatment

3.1 The results showed that the folk healers in Sa Kaeo Province used medicinal plants collected from their local community forests, which are found in every district. Community forests are therefore a source of abundance with medicinal plants used in malaria treatment by folk healers. These medicinal plants can be found mostly in Khok Sung District, Watthana Nakhon District, Ta Phraya District and Wang Nam Yen District. However, they are less found in Mueang Sa Kaeo District, Khlong Hat District, Khao Chakan District, Aranyaprathet District and Wang Sombun District. Additionally, it was found that certain locations, like the community forest in Watthana Nakhon District, there are irrigation systems in place to ensure the continuous growth of medicinal plants during the dry season.

Julian Steward (1955) developed the relevant and significant Cultural Ecology Theory, which explains how people interact with their surroundings and how they must adapt to the environment in each area. This is consistent with the concept of holistic health care. Holistic health care integrates traditional knowledge with complementary treatments to promote good health and prevent diseases. At present, holistic health care is being used to treat patients or people with illness, which is called holistic medicine. Well-being is having good health, happiness, balance, and holistic in 4 dimensions: physical, mental, social, intellectual or spiritual that is integrated in human and social development in order to achieve "peace and happiness". It is an interdependent relationship involving the environment, body, mind, and spirit. Achieving well-being requires a balance in this relationship. This is consistent with a study by Prangthip Buafueang (2014), who studied the application of geographic information systems (GIS) to study the distribution of dengue fever (DHF) outbreaks in the Tha Mai District, Chanthaburi Province, classified by person, time, and location using spatial data obtained from GIS.

Regarding the significance of the geographic information system, the results showed that it can be used to ascertain malaria-related variables such as malaria patient density, type of land use, Anopheles mosquito flight distance, rainfall, temperature, and relative humidity. It is very useful for understanding natural systems and changes resulting from interactions between humans and the environment (Benjaporn Srisombun and Chanadda Rattana, 2021, Anya Buchayan et al., 2018).

Recommendation

Practice Recommendation

The findings demonstrated that malaria patients along the Thai-Cambodian border continue to seek treatment from folk healers. As a result, the advancement of treatment techniques, the application of medicinal plants, and the restoration of herbal trade may serve as models for the actual growth of jobs for community members and strengthen the roles of herb sellers and folk healers. More importantly, as a result of globalization, we should encourage the restoration of folk medical knowledge in order to contribute economic and social value based on local knowledge bases and ways of thinking.

The results showed that more than 20 species of medicinal plants that are used to treat late-stage cancer can also be used to treat malaria. Therefore, private pharmaceutical companies and the pharmacy departments of all universities should conduct research and analysis on the active substances used in the treatment of malaria in order to produce medications at a lower cost for the public as an alternative treatment and for selling at both domestic and international level.

The findings indicated that in order to promote the growth of these medicinal plants and foster community understanding of sustainable development, the Ministry of Public Health should arrange for all relevant agencies to visit the locations of medicinal plants with coordinates specified in this geographic information system (GIS) and carry out conservation, restoration, and the development of a water system.

Further research Recommendation

1. Other 20 active substances in medicinal plants used in malaria treatment should be studied for potential pharmaceutical uses in the production of quality medicines.
2. Action research on the sustainable conservation and restoration of medicinal plants should be carried out with the help and participation of communities in Thailand and Cambodia to lessen and prevent disease outbreaks.
3. The location of medicinal herbs in protected forest areas should be studied in order to create a geographic information system (GIS), especially in large forests.

Declarations

Ethical Approval and Consent to participate

Not applicable

Funding Statement

Not applicable

Conflicts of interest

Not applicable

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