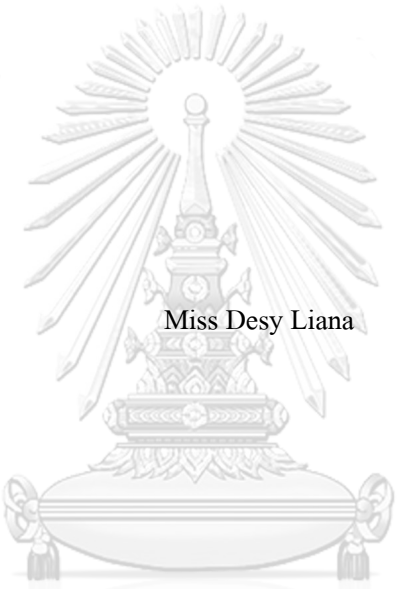
Phytochemical Screening, *In Vitro* Antimalarial Activity and Genetic Diversity of Selected Asteraceae Medicinal Plants Indigenous to Thailand

Miss Desy Liana



A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Public Health Sciences

Common Course

COLLEGE OF PUBLIC HEALTH SCIENCES

Chulalongkorn University

Academic Year 2020

การตรวจสอบทางพฤกษเคมีเบื้องต้น ฤทธิ์ต้านมาลาเรียในหลอดทดลองและความหลากหลายทางพันธุกรรมของพืชสมุนไพรบางชนิดในวงศ์ทานตะวันที่พบในประเทศไทย

น.ส.เดซี่ เลียน่า

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์สาธารณสุข ไม่สังกัดภาควิชา/เทียบเท่า
วิทยาลัยวิทยาศาสตร์สาธารณสุข จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2563

Thesis Title	Phytochemical Screening, *In Vitro* Antimalarial Activity and Genetic Diversity of Selected Asteraceae Medicinal Plants Indigenous to Thailand
By	Miss Desy Liana
Field of Study	Public Health Sciences
Thesis Advisor	KANCHANA RUNGSIHIRUNRAT

Accepted by the COLLEGE OF PUBLIC HEALTH SCIENCES, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the COLLEGE OF PUBLIC HEALTH SCIENCES

()

THESIS COMMITTEE

..... Chairman

(SATHIRAKORN PONGPANICH)

..... Thesis Advisor

(KANCHANA RUNGSIHIRUNRAT)

..... External Examiner

(Wanna Chaijaroenkul)

เดชี่ เลียน่า : การตรวจสอบทางพฤกษเคมีเบื้องต้น
ฤทธิ์ต้านมาลาเรียในหลอดทดลองและความหลากหลายทางพันธุกรรมของพืชสมุนไพร
บางชนิดในวงศ์ทานตะวันที่พบในประเทศไทย. (Phytochemical Screening, *In Vitro* Antimalarial Activity and Genetic Diversity of Selected Asteraceae Medicinal Plants Indigenous to Thailand) อ.ที่ปรึกษาหลัก : กาญจนา รังษีหิรัญรัตน์

การดื้อต่อยาอาร์เทมิซินินทำให้เกิดความพยายามในการค้นหายาต้านมาลาเรียชนิดใหม่ การใช้ข้อมูลการวิวัฒนาการร่วมกับข้อมูลทางพฤกษศาสตร์ในการค้นหาพืชที่มีฤทธิ์ต้านเชื้อมาลาเรียอาจเป็นประโยชน์ในการทำนายฤทธิ์ทางชีวภาพและลดค่าใช้จ่ายและเวลาในการวิจัยในห้องปฏิบัติการ วัตถุประสงค์ของการศึกษานี้คือการตรวจหาฤทธิ์ต้านเชื้อมาลาเรียสารพฤกษเคมีเบื้องต้นและศึกษาความหลากหลายทางพันธุกรรมของพืชสมุนไพรบางชนิดในวงศ์ Asteraceae ที่ได้จากข้อมูลการวิวัฒนาการร่วมกับข้อมูลทางพฤกษศาสตร์

จากการทบทวนวรรณกรรมสมุนไพรจำนวน 733 ชนิด พบว่ามีเพียง 340 ชนิดเท่านั้นที่มีคุณสมบัติตรงตามเกณฑ์การคัดเข้าเพื่อใช้ในการศึกษาลำดับเบสของ Internal Transcribed Spacer (ITS) ที่ได้จากฐานข้อมูล NCBI เมื่อนำมาวิเคราะห์การเรียงลำดับเบสด้วยโปรแกรม MUSCLE และ ITOL เพื่อสร้างแผนภูมิต้นไม้และจัดกลุ่ม พบว่าสมุนไพรที่ใช้ในการรักษามาลาเรียอยู่ในวงศ์ Asteraceae, Apocynaceae, Rubiaceae และ Euphorbiaceae โดยพบมากในกลุ่ม Asteraceae

สารสกัดเอทานอลของพืชสมุนไพรไทยในวงศ์ Asteraceae จำนวน 16 ชนิดถูกคัดเลือกมาเพื่อตรวจสอบทางพฤกษเคมีเบื้องต้น ฤทธิ์ต้านมาลาเรียและความหลากหลายทางพันธุกรรม การตรวจสอบทางพฤกษเคมีเบื้องต้นด้วยวิธีมาตราฐานเพื่อตรวจหาสารอัลคาลอยด์ ฟีนอลิก ฟลาโวนอยด์ ไตรเทอร์พีน สเตียรอยด์ ซาโปนิน ไดเทอร์พีนและแลคโตนการทดสอบฤทธิ์ต้านมาลาเรียในหลอดทดลองด้วยวิธี DNA fluorescence-based assay ต่อเชื้อมาลาเรียชนิดฟัลซิพารัมสายพันธุ์ 3D7 จากฤทธิ์ต้านมาลาเรียด้วยค่า IC_{50} (µg/mL) สำหรับการศึกษาหลากหลายทางพันธุกรรม จากการสกัดดีเอ็นเอของพืชสมุนไพรด้วยวิธี CTAB ทำการเพิ่มปริมาณสารพันธุกรรมหาลำดับเบสโดยใช้ ITS ไพรเมอร์ เปรียบเทียบลำดับเบสด้วยโปรแกรม MAFFT และวิเคราะห์แผนภูมิต้นไม้ด้วย ITOL และ Adobe Illustrator 2020 โดยมี *Cannabis sativa* เป็นตัวเปรียบเทียบ

พืชสมุนไพรที่สกัดด้วยเอทานอลทั้ง 16 ชนิด พบว่ามีสารฟีนอลิกและฟลาโวนอยด์ จากผลการทดสอบฤทธิ์ต้านมาลาเรียในหลอดทดลองในพืชสมุนไพร 16 ชนิด พบว่า 8 ชนิดมีฤทธิ์ต้านมาลาเรียระดับต่างกัน ได้แก่ ระดับดีถึงปานกลาง 1 ชนิด (*Sphaeranthus indicus*) ระดับน้อย 4 ชนิด (*Blumea balsamifera, Artemisia chinensis, Artemisia vulgaris, Tridax procumbens*) และระดับน้อยมาก 3 ชนิด (*Wedeliatrilobata, Eupatorium capillifolium, Vernonia cinerea*) ส่วนสารสกัดที่เหลืออีก 8 ชนิดพบว่าไม่มีฤทธิ์ต้านเชื้อมาลาเรียสายพันธุ์ 3D7 สารสกัดที่มีฤทธิ์ดีที่สุดคือส่วนของลำต้นเหนือดินของ *Sphaeranthus indicus*มีค่า IC_{50} 6.59 µg/mL จากแผนภูมิต้นไม้โดยใช้ลำดับเบสของ ITS สามารถจัดจำแนกสมุนไพรที่ศึกษาออกเป็นกลุ่มๆ กล่าวโดยสรุป การศึกษาทางวิวัฒนาการนั้นมีประโยชน์ในการจำกัดการคัดเลือกกลุ่มสมุนไพรให้แคบลงเพื่อใช้ในการศึกษาฤทธิ์ทางชีวภาพ

สาขาวิชา	วิทยาศาสตร์สาธารณสุข	ลายมือชื่อนิสิต .....
ปีการศึกษา	2563	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6278001453 : MAJOR PUBLIC HEALTH SCIENCES

KEYWORD: antimalarial phytochemicals genetic diversity Plasmodium falciparum phylogeny ethnobotany

Desy Liana : Phytochemical Screening, *In Vitro* Antimalarial Activity and Genetic Diversity of Selected Asteraceae Medicinal Plants Indigenous to Thailand. Advisor: KANCHANA RUNGSIHIRUNRAT

The emergence of artemisinin resistance leaded the effort to find the new antimalarial drug or artemisinin activity booster. Due to the chance that secondary metabolites can be evolutionary conserved, combining phylogeny with ethnobotanical data for screening antimalarial activity may be helpful to predict bioactivity and minimize the expenditure and time for laboratory research. The aimed of this study is screening the antimalarial activity, phytochemicals and genetic diversity of selected Asteraceae medicinal plants generated by combinatorial phylogeny and ethnobotanical data.

733 medicinal plants were obtained from literature search however only 340 taxa were met the inclusion and exclusion criteria hence these taxa were further analysis. Obtained 340 Internal Transcribed Spacer (ITS) sequences from gene bank NCBI were analyzed by MUSCLE sequence alignment and Maximum Likelihood Phylogenetic Test to generate the phylogenetic tree. Interactive Tree of Life (ITOL) was used to analyze the clustered pattern in generated phylogenetic tree. Several clades were highlighted consistently in the phylogenetic tree for malaria treatment including Asteraceae, Apocynaceae, Rubiaceae and Euphorbiaceae while the strong signal was majorly shown in Asteraceae.

Afterwards, 16 ethanolic extract of Thai Asteraceae medicinal plants were investigated to determine the phytochemical screening, antimalarial activity, and the genetic diversity. Alkaloids, phenolics, flavonoids, triterpenes, steroids, saponins, diterpenes and lactones were screening by standard method. Antimalarial activity assay was done by DNA fluorescence-based assay against laboratory adapted 3D7 *Plasmodium falciparum*. Classification of antimalarial activity was done by categorizing the IC_{50} (µg/mL). In other hand, the genetic diversity was examined. Extracted plant DNA by CTAB method was amplified and sequenced by universal ITS primer. Phylogenetic analyses were performed with *Cannabis sativa* as outgroup species. MAFFT sequences alignment and RAxML with automatic bootstrapping were performed to generate phylogenetic tree followed by making up with ITOL and Adobe Illustrator 2020.

All 16 ethanolic extract medicinal plants showed the presence of phenolics and flavonoids. Among 16 medicinal plants tested, 8 showed active which exhibited good-moderate (1; *Sphaeranthus indicus*), weak (4; *Blumea balsamifera, Artemisia chinensis, Artemisia vulgaris, Tridax procumbens*) and very weak (3; *Wedelia trilobata, Eupatorium capillifolium, Vernonia cinerea*) and the 8 remaining extract were showed inactive. The best promising extract is *Sphaeranthus indicus* with the IC_{50} 6.59 µg/mL. Constructed phylogenetic tree using ITS region showed to be able to separate the species into their clade tribe based on current classification. In conclusion, phylogeny approach is useful to narrow down the selection of candidate taxa for bioactivity screening.

Field of Study:	Public Health Sciences	Student's Signature .....
Academic Year:	2020	Advisor's Signature .....

ACKNOWLEDGEMENTS

would love to express my sincere gratitude to my beloved advisor, Assoc. Prof. Kanchana Rungsihirunrat who guide me with the full of patience on both thesis and academic issues during my master study. I could not finish my thesis writing without her continuous helps, immense knowledge, kind guidance, encouragement and nice motivation which were kept me stay mentally healthy. I am very thankful to have her during my master study.

Besides, I am grateful to my thesis committees: Prof. Sathirakhorn Pongpanich, Assoc. Prof. Wanna Chaijaroenkul for their valuable comments, advices and questions which were build up my knowledge.

My deepest thanks to Assoc. Prof. Wanna Chaijaroenkul and Dr. Matthew Phanchana for their kindness support hence I could learn and finished the antimalarial assay. I would like to express my sincere thanks also to Assoc. Prof. Chanida Palanuvej, Dr. Anuchit Phanumartwiwath and P'Somchai for their kindness supports, guidances and insightful discussions during developing of phytochemical screening method.

For all my professors in Public Health Sciences: Assoc. Prof. Kanchana Rungsihirat, Assist. Prof. Naowarat Kanchanakhan, Dr. Tepanata Pumpaibool, Assoc. Prof. Chanida Palanuvej, Dr. Anchalee Prasansuklab, Dr. Anuchit Phanumartwiwath and Dr. Onuma Zongram, I would express my sincere thanks for their invaluable advice during developing and doing my research.

For the staff and my fellow lab-mates in Biomolecular Laboratory, P' Napaporn, P' Kannittha and P' May Myath Thu, thank you for always supporting, accompany and cheer me up during my up and down in experimental study. My sincere thanks to all my friends in Public Health Sciences Program (Irfanul Chakim, P' Boonyarat, P' Mint, P' Pare, Phimkun, Sathakun, P' Alison, P' Akarat, P' Ploychat, P' Meen) and for all my Indonesian friends in Public Health Program (Nurnaningsih Herya Ulfah, Lafi Munira, Syarifah Aqilah, Ridwan Fauzi, Siti Syahidati Fauzana and Fena Amalia Untari). Also, I would like to express my sincere thanks to all my friends who could not be stated one by one here.

My sincere gratitude to all professors and supporting staffs of College of Public Health Sciences, Chulalongkorn University for their insightful lectures, sharing and technical supports. And also, I would like to acknowledge AEC Educational Scholarship and 90th Anniversary Research grant from Chulalongkorn University hence I could achieve to study here.

At last, foremost, I am extremely grateful to my parents, my sisters and brother who always pray for me and support me from miles away hence I could survive and happy studying here.

Desy Liana

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I INTRODUCTION

1.1. BACKGROUND AND RATIONALE

Malaria is a vector borne infectious disease caused by protozoan parasites called *Plasmodium,* transmitted by female *Anopheles* mosquito. This disease still became major challenge in the health problems. WHO reports that there are 228 million cases occured in 2018 and 405.000 people death because this disease[1]. Nowadays, there are 5 species of *Plasmodium* which can infect human such as *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesii*. However, *P. falciparum* is the deadly one among the others [2].

From ancient time, plant has been used to treat malaria in various region around the world. In 1820, the first antimalarial drug (alkaloid quinine) was extracted from bark *Cinchona* (Rubiaceae) and become the core of malaria treatment but after that the usage was suspended by chloroquine. This chloroquine drug was successful for malarial treatment but then resistance has been occurred afterwards [3]. After the spread of resistant to the chloroquine and other synthetic drugs, artemisinin then replacing the core treatment for malaria disease [4]. Artemisinin is antimalarial drug which derived from Chinese plant “qinghao” (*Artemisia annua*). Currently, artemisinin and its derivatives are being used as the first and second-line treatment for malaria [2].

Artemisinin based combination therapies (ACTs) is the first and second line treatment for uncompleted *P. falciparum* and chloroquine resistance *P.vivax* which are recommended by WHO. This drug and derivatives able to reduce the number of parasites during the first 3 days of treatment while the partner drug is functioning to eliminate the remaining parasites [1]. Artemisinin has fast action and shorter time for clearance the parasite compare to other malarial drugs and only active in the bloodstage parasites. Artemisinin act quickly and can kill every asexual red blood cell stage. The rapid action made artemisinin derivatives effective against severe malarial. However, this drug also disappeared quickly hence recrudescences may be occurred when using monotherapy[5].

Unfortunately, the emergence of *P. falciparum* resistance to ACTs including artemisin derivative and their partner drug has occurred. In 2008, reduced clinical efficacy of ACTs was reported in Western Cambodia (Thailand- Cambodia border area) which showed the delayed clearance of the parasites. Resistance to artemisin has spread over in Southeast Asia. In 2017, artemisin resistance was limited in the Greater Mekong subregion (Cambodia, Thailand, Myanmar, Lao PDR, Vietnam, Myanmar-China-India border [6]. One of the solutions for this case is discovery of new drug of antimalarial which could be act as replacement of artemisinin or artemisinin activity booster.

Emergence of resistance is the major challenge for malaria eradication mission. Various strategies are laid down by World Health Organization such as vector control, source reduction, early case detection, prompt treatment, development of new diagnosis and vaccines, nevertheless the need for new and efficacious drugs has become critical priority [7]. World Health Organization (WHO) reported that about 65-80% people where live in developing country depend inessentially on plant for primary health care. Herbal material has been used since a long time ago as a medicine for treatment or prevention of numerous of disease. Over the past decade, interest in drug derived from plant has increased expressively which about 25% of modern medicines are derived from plants[8]. A lot of drugs have been developed from plant natural products due to its bioactive compounds (e.g. alkaloids, terpenoids, phenolics) which well known to have therapeutic properties.

Targeted approach in natural product research for drug discovery can be based on ethnobotanical and chemo-taxonomical data. Local people's wisdom of knowledge and experience from ancient years ago about using various herbs for treating the disease may become a tool for discovery new promising agent. Moreover, similar group of compounds can be expected to be found under one genera or family.

Ethnobotanical bioprospecting has contributed in drug discovery generating various current modern drug including antimalarial. However, merely using ethnobotanical directed bioprospecting may encountered several disadvantages. Placebo effects may be occurred in traditional medicine practices. Additionally, there are still many traditional herbal medicines have not been tested or have tested but showed none

or less efficacy. Furthermore, there are no robust methods which suggest for selecting the plants for further bioactivity screening effectively in purpose to avoid exhaustive testing. Phylogenetic approach becoming a new prospective tool to predict the power of traditional medicine whereas several studies showed that related medicinal plants species are used by local people in the different region to treat medical condition in the same therapeutic areas. This approach also may be useful for discovering new candidate plant which never been used in ethnomedicine. The study by combining phylogeny with ethnobotanical data from three different biodiversity hotspot area including Nepal, South Africa Cape and New Zealand showed that medicinal plant showed to be pretty concentrated in certain sites of the constructed phylogenetic trees, were not scattered randomly [9]. Based on this finding, some suggest that the exploration for looking new medicinal plant for drug discovery should begin from the "hot" groups which were assumed to share similar phytochemical or bioactivity power [10].

Phylogeny or relatedness among species can be construct using the heritable characters. The characters may be morphological, chemical or genetical. Conventionally, the phylogenetic trees are constructed by physical examination. Nowadays, molecular analysis has been used to refine or support the constructed phylogenetic tree using DNA sequence of particular-gene or DNA region to examine the similarities. DNA barcoding can be performed using various marker including Internal transcribed spacer (ITS) region. ITS region is ribosomal DNA which occurred as arrays of tandem repeats and dispersed in the various locations in the genome [11]. Despite ITS, there are common nuclear sequences which being used for plant DNA barcoding such as plastid genes (e.g. *matK, rbcL, psbA-trnH, trnL* intron, etc) and intergenic spacer (IGS). However, ITS region showed high authentication efficiency due to the length and sequence and have relatively high mutation and evolution rates [12] hence this region widely used as a marker because of its high resolution of intra- and interspecific relationships [13]. Furthermore, this region is easy to amplify using universal primer [14].

The well-known herbs *Artemisia annua* ("qinghao"), which produce sesquiterpene lactone artemisinin is belong to Asteraceae family [15]. Asteraceae is the largest flowering plant families which have members over 900 genera and 14.000 species

[16]. Several species in this family has being used by local people in various region of the world for treating malaria and fever traditionally.

Thailand is relatively small country but well known on its richness in the biodiversity including plant diversity. Moreover, Thai people have used the plants as a sources or traditional medicine for making the remedies of ailments in such of a long history.

1.2. RESEARCH GAP

Due to the emergence of artemisinin resistance, new candidate drug for malarial become urgently needed. Ethnobotanical study may be a tool to lead the drug discovery due to experience and knowledge of the people from ancient time whereas preliminary screening of bioactive compound is useful for guiding the further research in drug discovery derived from plants. Exhaustive testing for discovery new candidate drug from ethnobotanical studies was trigger people to discover new approach to looking for the more effective way. Phylogeny among species which can be constructed using molecular marker become a new candidate tool to predict the power of traditional medicine due to the possibility of sharing similar bioactivities and chemical characters between related species. However, oversimplify for making the conclusion regarding to this approach should not be done. Until this time, this relation has rarely been tested hence investigation of this approach is needed in order to observe the pattern between the phylogeny, phytochemical diversity and the power of bioactivity.

There is no reported study which investigate *in vitro* antimalarial activities, phytochemical and genetic diversity in the population of indigenous Thailand's medicinal plants generated by combinatorial phylogeny and ethnobotanical data approach. Furthermore, this study is first attempt which investigate the relationship between phylogeny and the diversity of the phytochemical and the antimalarial activity of the selected medicinal plants.

1.3. RESEARCH QUESTIONS

1.3.1. How does the clustered pattern of ethnobotanical plants used for malaria treatment in the constructed phylogenetic tree?

1.3.2. How are the profiles of the bioactive compounds of selected Asteraceae medicinal plants indigenous to Thailand?

1.3.3. How does the *in vitro* antimalarial activities of selected Asteraceae medicinal plants indigenous to Thailand?

1.3.4. Which plant showed promising antimalarial activity among all tested plants?

1.3.5. How are the patterns of ITS sequence among all plant species tested?

1.3.6. Is there any pattern or relation between phylogeny with the phytochemical diversity and the power of bioactivity from antimalarial assay?

1.4. OBJECTIVE

1.4.1. GENERAL OBJECTIVE

To investigate the phytochemical screening, *in vitro* antimalarial activities, and genetic relationship of selected medicinal plants indigenous to Thailand generated from combinatorial phylogeny and ethnobotanical data approach.

1.4.2. SPECIFIC OBJECTIVES

1.4.2.1. Investigate the clustered pattern of medicinal plants used for malaria and its associated symptoms by combining phylogeny and ethnobotanical data.

1.4.2.2. Determine the presence of secondary metabolites in crude extract using standard phytochemical screening of each selected Asteraceae medicinal plant indigenous to Thailand.

1.4.2.3. Determine the antimalarial activity of each selected Asteraceae medicinal plant indigenous to Thailand.

1.4.2.4. Determine the promising plant among all plant species tested.

1.4.2.5. Determine the ITS sequence of all plant species tested.

1.4.2.6. Investigate any pattern or relation in phylogeny on their phytochemical diversity and the power of antimalarial activity among all tested plants.

1.5. CONCEPTUAL FRAMEWORK

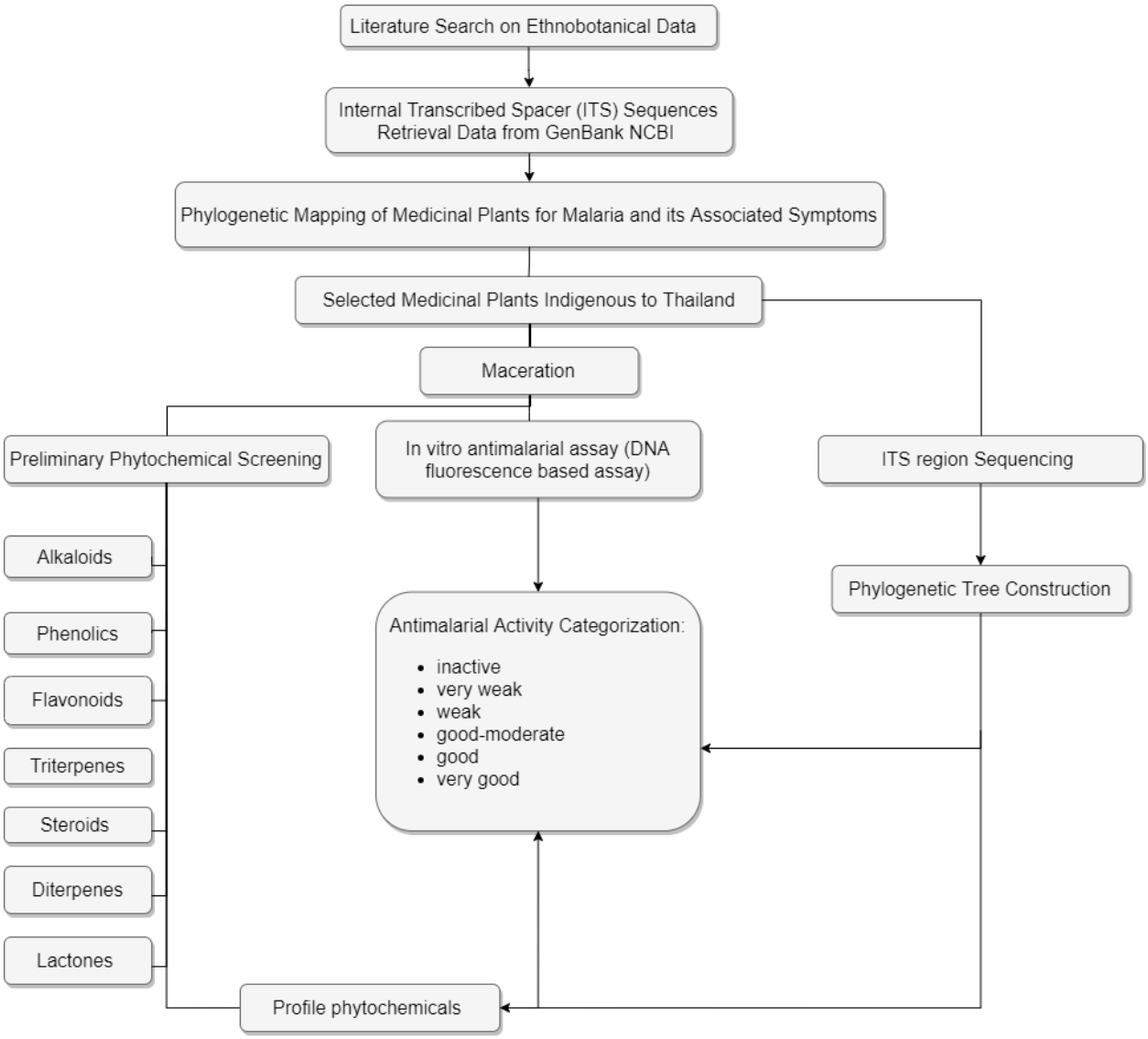


Figure 1 Conceptual framework

CHAPTER II LITERATURE REVIEW

2.1. MALARIA

Malarial is infectious diseases cause by protozoan parasites, *Plasmodium*. There are 5 *Plasmodium* species which can infect human (P. *falciparum*, *P. vivax, P. ovale, P. malariae and P. knowlesi*). However, *P. knowlesi* is known as causative agent of zoonotic disease because the reservoir host is not human but long tailed *Macaca* monkey.

Major human malarial are caused by *P. falciparum* and *P. vivax* while *P. falciparum* is more virulent and cause severe malarial anemia. It can induce anemia during their blood stages of infection [17]. *Plasmodium* species is a protozoan parasite belong to Phylum Apicomplexa, Class Aconoidasida, Order Haemosporida and Family Plasmodiidae [18]. Among of *Plasmodium* species, *P. falciparum* known to has ability to progress rapidly to severe illness or death. This species predominates in Papua New Guinea, sub-saharan Africa and Hispaniola [19].

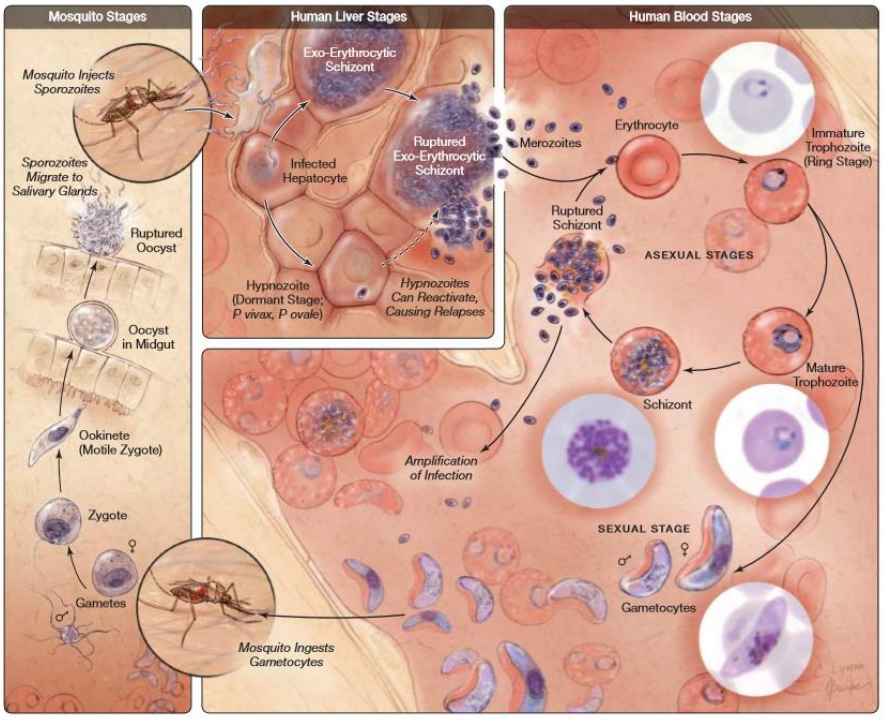


Figure 2. Life cycle of P. falciparum parasites [19]

During blood feeding of female *Anopheles* mosquito, sporozoites are injected into the skin then invading the hepatocytes [18]. Sporozoite which infect liver cell will mature into schizont. This schizont can rupture and release number of merozoites. There is an exception in *P. vivax* and *P. ovale* which can persist in a dormant stage, a hypnozoite. The dormant hypnozoites can cause relapse and release in the bloodstream after a week or years later. Released merozoites then infect red blood cells and then turn into trophozoites. The ring stage (immature tropozoites) will mature and turn into schizont stage which can produce and releasing merozoites when rupture. Disease is caused by this asexual blood stages. The cycle repeated when microgametocyte and macrogametocyte are ingested during a blood meal. Multiplication in the body of mosquito is called sporogony cycle. Microgamete will penetrate the macrogamete in the stomach and produce zygotes. These zygotes will turn into ookinetes which elongated and have ability to mobile. Ookinetes will invade the midgut wall and then will turn into oocysts which can develop later become sporozoites [20].

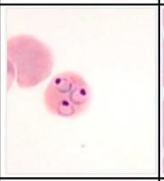
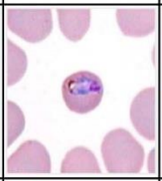
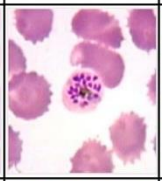
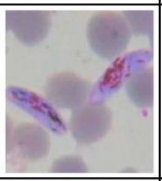
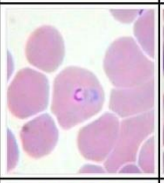
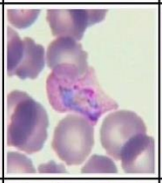
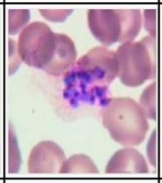
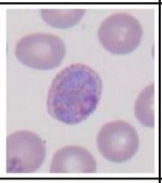
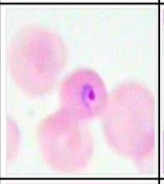
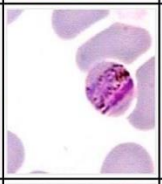
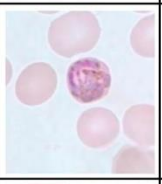
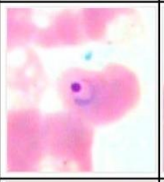
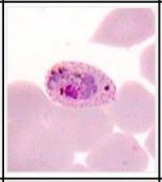
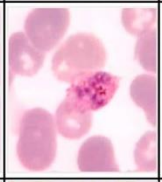
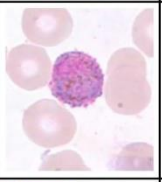
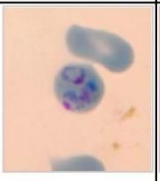
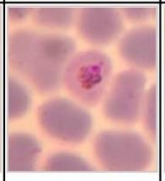
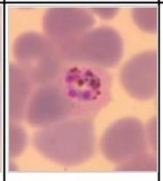
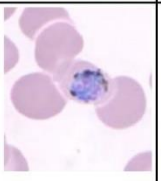
Human Malaria					
Stages / Species	Ring	Trophozoite	Schizont	Gametocyte	
P. falciparum					• *Parasitised red cells (pRBCs) not enlarged.* • *RBCs containing mature trophozoites sequestered in deep vessels.* • *Total parasite biomass = circulating parasites + sequestered parasites.*
P. vivax					• *Parasites prefer young red cells* • *pRBCs enlarged.* • *Trophozoites are amoeboid in shape.* • *All stages present in peripheral blood.*
P. malariae					• *Parasites prefer old red cells.* • *pRBCs not enlarged.* • *Trophozoites tend to have a band shape.* • *All stages present in peripheral blood*
P. ovale					• *pRBCs slightly enlarged and have an oval shape, with tufted ends.* • *All stages present in peripheral blood.*
P. knowlesi					• *pRBCs not enlarged.* • *Trophozoites, pigment spreads inside cytoplasm, like P. malariae, band form may be seen* • *Multiple invasion & high parasitaemia can be seen like P. falciparum* • *All stages present in peripheral blood.*

Figure 3. Blood stage (ring, trophozoite, schizont) of *Plasmodium* [21]

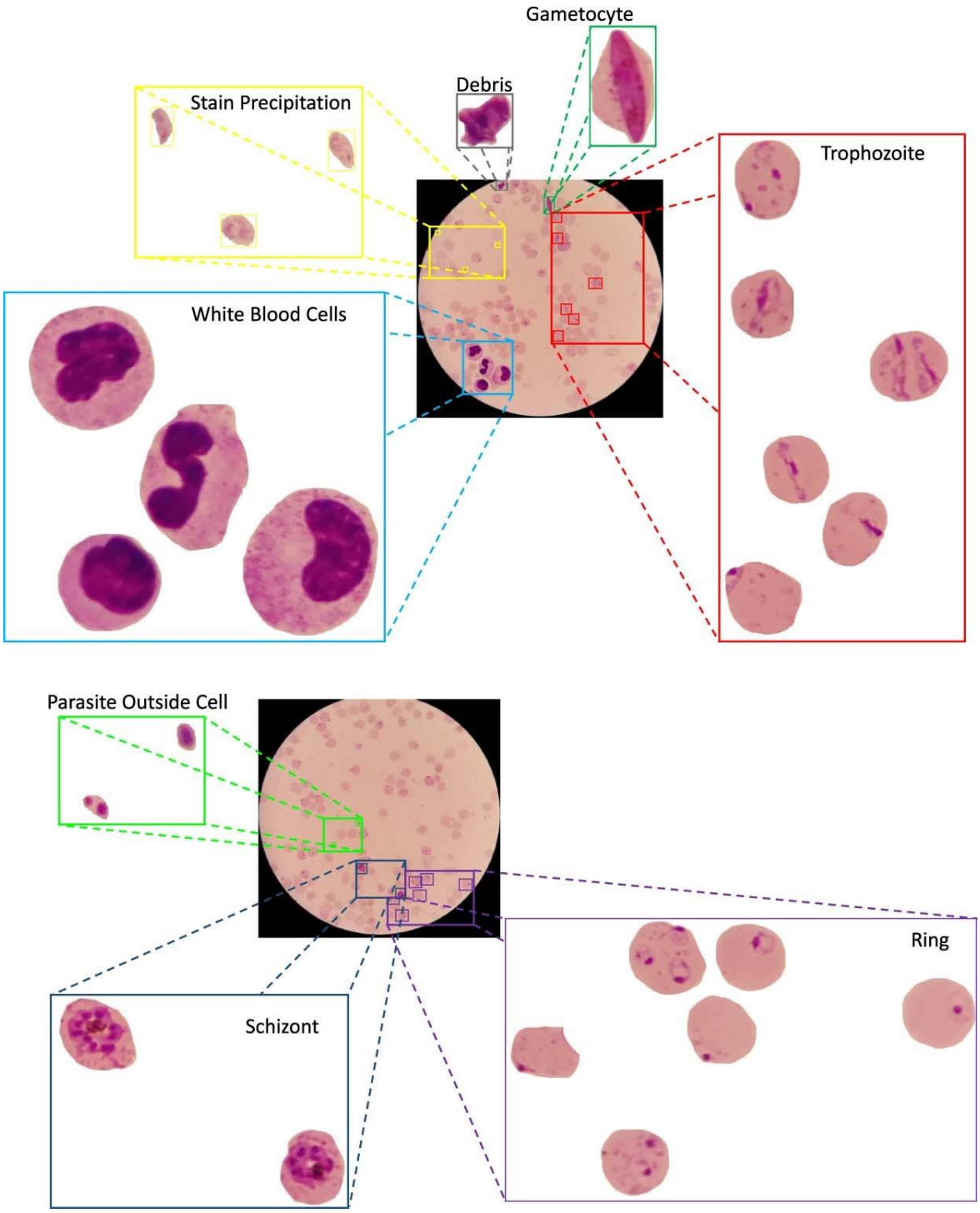


Figure 4. Parasite life stage in the single red blood smear [21]

Period of incubation varies between 7 and 18 days for *P. falciparum, P. vivax, and P. ovale*. In other hand *P. malaria*e show longer which is 18-40 days. The most common reported symptoms are fever and can be followed by other symptoms such like chills,

headache, dizziness, back pain, myalgias, cough, weakness, abdominal pain, or coma which may occur in weeks or even months after the infection. These symptoms can be occurred because of inadequate treatment or as a response to immunity. Severe malaria is characterized by one or more of the symptoms and mostly caused by *P. falciparum* [19].

Diagnosis is done according to patient's symptoms and physical examination. However, in the first patient showed vary and very non-specific symptoms. Delaying diagnosis and treatment can cause of death hence the rapid diagnosis can decrease the rate of case transmission. Thin and thick peripheral blood smears usually are conducted in the laboratory for diagnosis. In other hand, rapid diagnostic tests are also available (e.g. ICT, OptiMAL, Para-HIT-f, ParaScreen, Paracheck, SD Bioline and molecular technique such as polymerase chain reaction (PCR)) [22].

Nowadays, ACTs is the first and second-line treatment for uncomplicated malaria infection which is recommended by WHO. Treatment with this therapy is helpful to saving the life. The more problem to eradicate this disease is because of the emergence of resistance in all available antimalarial drug in the field [18].

2.2. HISTORY OF ANTIMALARIAL DRUG

2.2.1. Quinine

Quinine is derived from various *Chincona* species originating from South America which has been used traditionally to treat fevers including malaria fever since about 1630. After that, the introduction of more effective drug, chloroquine, has replaced the usage of quinine [4]. In the 18th century, *Chincona* has begun popular then lead the extinction of several species. In 1820, Pierre Pelletier and Joseph Caventou, young French Chemist did successfully isolate the active compound quinine. Afterwards, for more than a century, this compound became the only effective antimalarial available in the world. Mechanism of action of quinine is known to inhibit the formation of haemozoin because this compound can form a complex with the haem. The haem is toxic to the parasite which can cause the death of parasite [4, 23]. In 19th century the world's supply of quinine has been monopoly by the Dutch plantation in Java and quinine became the standard treatment for

intermittent fever for about mid-19th century to 1940s. After introduction of chloroquine and other synthetic antimalarials drug, the use of quinine has declined [23].

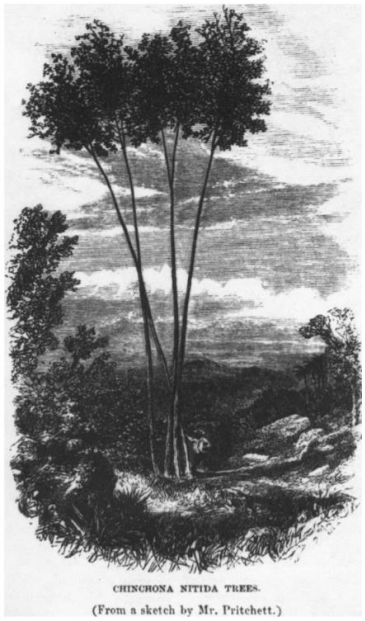


Figure 5. *Chincona* tree

[23]

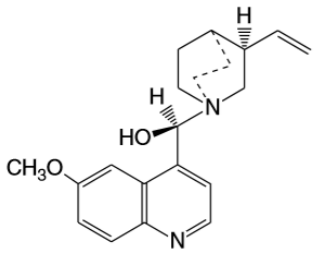


Figure 6. Structure of quinine

[4]

After emergence *P. falciparum* resistance due to the use of chloroquine and other antimalarials, people planned to use back quinine for chloroquine resistant malaria but then artemisinin has replacing the core treatment [4].

2.2.2. Synthetic Antimalarials

In 1856, an 18 years old Chemist, William Henry Perkins, planned to synthesize quinine but did not succeed. However, he was able to synthesize the first textile dye "mauve" which was persist and could not be wash with the water. This successfulness led the development of industry of synthetic dye and then triggered the advance of medicine. After that, the microbiologist used this synthetic dye to stain the microbial for their study. German scientist, Paul Ehrlich using methylene blue to stain malarial parasites and noticed that the parasite took this stain intensely. He assumed that the stain may be toxic to the parasites. Afterthat, in 1891, he cured two malarial patients using this stain. It was become the first synthetic drug for malaria ever which used for human treatment. After that, Bayer, the leading chemical company, immediately became the leading pharmaceutical company. Methylene blue was used as a prototype to developed new synthetic antimalarial drug. In 1925, they synthesized the plasmoquine or pamaquine which was effective against *P. vivax* and mepacrine or atebrine which was active to *P. falciparum* [23].

When Japanese took over Java during World War II, the supply of quinine was cut off and then plasmoquine and mepacrine were widely used. Then, the allied scientist from American, British and Australia was doing experimental through trial and error in purpose to discover new antimalarial drug. They synthesized and tested 16.000 compound and afterward discovered the chloroquine which known to be the powerful antimalarial was superior compared to atebrine. Furthermore, this drug became the most important drug for malaria during that time [23].

In 1950s, strategy to distribute the chloroquine drug in wider scale was done by using Pinnoti's method. People put the chloroquine into the common cooking salt which known as medicated salt program. This strategy has been introduced in Brazil by Mario Pinnoti and was applied in South America, Africa and Asia. At that time, treatment with chloroquine (CQ) or CQ-medicated salt was an important as a complement of malaria eradication program. However, the use of chloroquine was only curtailed in the beginning of 1960s because of the spreading of *P. falciparum* chloroquine resistant. This may be happened partly by medicated salt program. Afterwards, people developed and

introduced various synthetic drugs (e.g. primaquine, tafenoquine, pyrimethamine, sulfadoxine, mefloquine and atovaquone) [23].

2.2.3. Artemisinin and derivatives

Artemisinin is the sesquiterpene lactone isolated from the Chinese plant Qing Hao. For at least 2000 years, Qing hao (*Artemisia annua*, Asteraceae) is known to be Chinese herbal medicine for treatment of hemorrhoids. However, in 1596, the herbalist Li Shizzen, recommended this plant to be soaked in the cold water then applied to treat fever. Qing Hao was tested in the program of drug screening activity from traditional herbal medicine [23]. Afterthat, in 1972, scientist has discovered the active substance, known as qinghaosu (name later as artemisinin). This substance was shown to be highly potent to *P. falciparum* and showed to be effective against CQ-resistant and cerebral malaria in clinical trial. However, unfortunately, after first month of treatment, recrudescence has occurred because the drug could not kill all parasites. Due to this, then the drug is derivatized to improve their characteristics regarding to formulation strategy. Derivatization was done by reduction of lactone carbonyl to form dihydro-artemisinin and followed by making ether or ester derivatives [4].

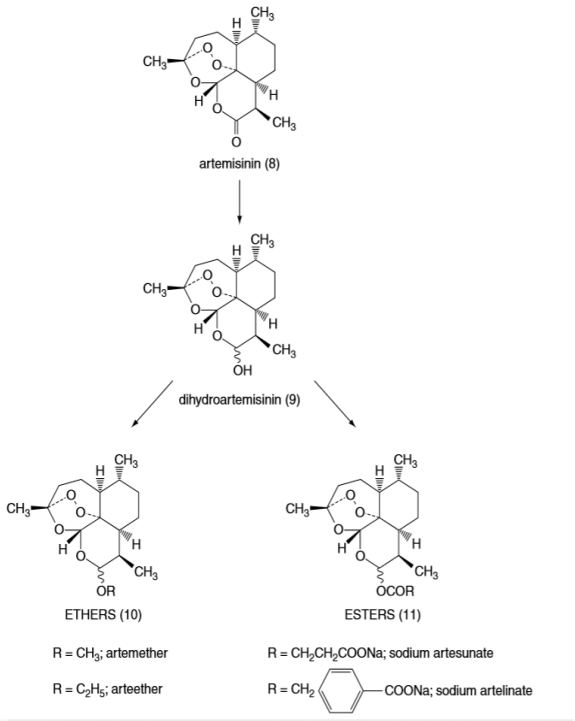


Figure 7. Structure of artemisinin and its derivatives

[4]

The ether, artemether is soluble in oil while the esters, sodium artelinate or sodium artesunate are water soluble. Artemether is given by intramuscular injection, whereas those two esters are given by intravenous injection or orally. According to regulation by WHO, artemisinin and its derivatives are not allowed to be used as monotherapy to prevent development of resistance. Artemisinin derivatives will be metabolized to active dihydro-artemisinin in the body [4].

Artemisinin and its derivatives which have 1,2,4-trioxane structure and its endoperoxide bridge known to be played in the mechanism of action against the parasites [24]. Malarial parasites are known to uptake the hemoglobin extensively and digest them during the erythrocytic stage. This activity makes a releasing number of active heme and free Fe^{2+}. Iron from heme is strongly activate the artemisinin and the endoperoxide bridge of artemisinin structure react with the iron haem and produce highly reactive free radicals which can attack parasite molecules such as proteins and nucleic acid. Artemisinin also active to gametocyte hence may contribute to decrease the rate of

transmission [4, 24]. Artemisinin have ability to directly alkylating the cellular protein such as TCTP (translationally control tumor protein) and endoplasmic reticulum Ca^{2+} ATPase PfATP6 then cause the death of the parasite [24]. Artemisinin is stable in neutral organic solvent in temperature reach up to 150 ^{0}C, degrades during drying at 190 ^{0}C, fairly stable during exposure of light and heat but there is no report about in certain intensity [25].

2.3. EMERGENCE OF RESISTANCE

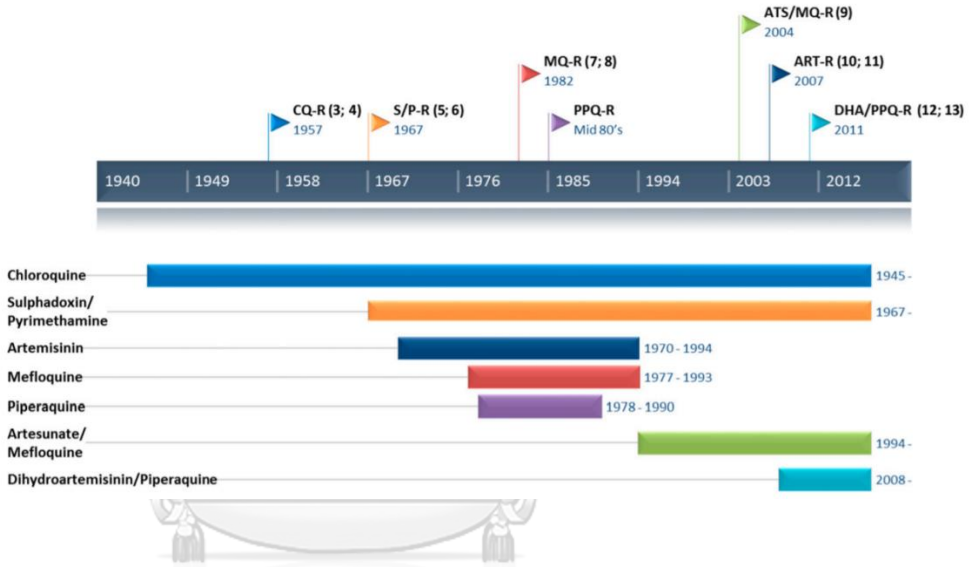


Figure 8. Timeline between introduction antimalarial drug and the first case of resistance *[26]*

ACTs is become standard core treatment for uncomplicated *P. falciparum*. The purpose of the use drug in combination is rational to prevent the occurrence of drug resistant. Artemisinin resistance is assumed to be unlikely happened because of its short half-life and quick action. Unfortunately, after first year recommendation, the sensitivity of *P. falciparum* toward artemisinin was reduced. First report was come from Thailand-Cambodia border. Since the first report, then a various report has come from the other region such as China, Equatorial Guiniea and Uganda Africa [27].

Artemisinin resistance is defined as the delayed clearance of the parasite more than 5 hours. In 2008, first partial *P. falciparum* resistance to artemisinin was reported at

Battambang, Western Cambodia and also other region such as southern Myanmar (Burma), western Thailand, southern Vietnam and China [26]. Resistance to artemisinin was observed in the ring stage of parasites which show the delayed clearance in the circulation. *pfkelch13* (K13) is the primary gene marker for the resistance [28]. Various study had confirmed the association of polymorphisms in the *P. falciparum* Kelch13 propeler protein. Nowadays, PfK13 mutation is carried by the parasites including N458Y, F4461, Y493H, M4761, R539T, 1543T, R561H, P553L and C580Y which have known to attribute the delayed clearance and decrease the sensitivity of the drug [27].

K13 protein is E3 ligase substrate adapter which bind to phosphatidylinositol-3-kinase (P13K) for proteosomal degradation. Mutation in *Kelch* 13 may result in decreasing the P13K proteolysis and increasing levels of lipid PI3. This can lead autophagy, unfolded protein response (UPR) can be accumulated response of stress which can lead survival of the parasites [27].

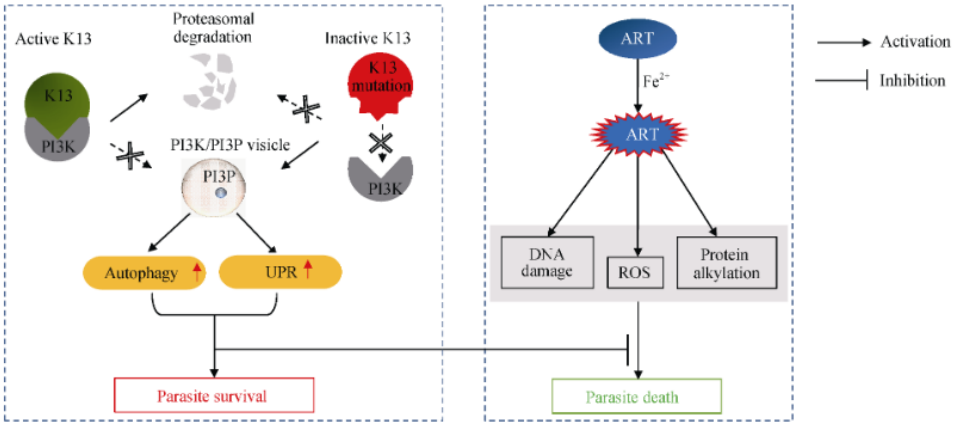


Figure 9. *P. falciparum* artemisinin resistance proposed mechanism. Ferrous ions activated the artemisinin (ARTs) within malarial parasites, lead the DNA damage, reactive oxygen species (ROS) production, protein alkylation and trigger the death of parasites. Kelch 13 (K13) mutation will decrease proteolysis of P13K (phosphatidylinositol-3-kinase) and increase lipid product PL3P which stimulate autophagy, engage the unfolded protein response and eventually promote the survival of the parasites [27]

Despite of that worrying condition, ACTs still be remained as the most effective treatment for uncomplicated falciparum malarial. According to WHO, treatment prescription is no required to be change radically as long as the complement drug in ACTs combination is effective continuously. Selection should be taken carefully to minimize the risk of multiply resistant strain. Compound discovery to overcome this artemisinin resistant recently has become more urgent [27].

2.4. PLANT NATURAL PRODUCT

Plant natural products have been widely known to have the therapeutic effect against various diseases or agent of diseases. The bioactivity of natural product is commonly from secondary metabolites. According to [29], secondary metabolites are metabolites which are not required in growth or maintaining function of the cells while primary metabolites necessary for "primary" function of plant growth, photosynthesis and reproduction. Secondary metabolism may be play in defend and survival mechanism to environmental stresses under attack of pathogen, predator or even other plant (allelochemic).

Primary metabolites are produced by all plants hence can be found in all plant (species, genera and family). The plant will synthesize the primary metabolites in their normal metabolic activities (e.g. carbohydrates, protein, amino acids and lipids) while the secondary metabolites are synthesized in such specialized cell at the various life stages. Secondary metabolites sometimes can be used as taxonomic characters [29]. A simple classification of secondary metabolites according to [29] including three main groups:

1. Alkaloids (nitrogen-containing compound)
2. Phenolics (constructed from simple carbohydrates contained benzene rings, oxygen and hydrogen)
3. Terpenoids (synthesized via mevalonate pathway, composed of hydrocarbon chain)

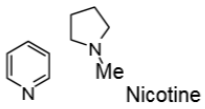
2.4.1. Alkaloids

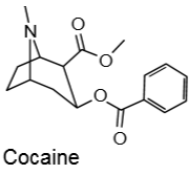
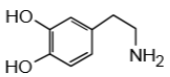
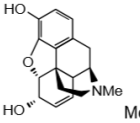
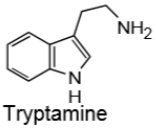
Alkaloid is one of the largest secondary and more than 10000 type of alkaloids have been identified from more than 300 families. Alkaloids is a nitrogenous molecule with conform complex ring structure which usually are derived from amino acids. Naturally, alkaloids can be found in all plant organ. Most of them have a bitter taste [30].

Generally, alkaloids contain one nitrogen atom which may exist as a primary amine (RNH_2), secondary amine (R_2NH) and tertiary amine (R_3N). The nitrogen atom contains an unshared pair of electrons. The compound is basic in nature and possess chemical properties of ammonia. Free amine will be free when hydroxide ion reacted with salts of alkaloids[31].

Based on biological pathway and their precursor, alkaloid can be divided into these following groups: true alkaloids, proto-alkaloids and pseudo-alkaloids. Pseudoalkaloids are not derived from amino acid but from precursors or post-cursors (derivatives and degradation process) of amino acids while the others are derived from amino acids. On other hand, protoalkaloids do not have nitrogen atom incorporated within the ring structure while the true alkaloids have. Cocaine, dopamine, quinine and morphine are examples of true alkaloids while mescaline, hordenine and yohimbine are protoalkaloids. Coniine, capsaicin, ephendrine, solanidine, caffeine and theobromine are such examples of pseudoalkaloids[30]. According to [29], alkaloids also can be classified based on the precursor of amino acids:

Table 1. Alkaloids classes

No	Alkaloids Class	Description	Examples and structures
1	Pyridine and piperidine	This group is representing a class of compound which have effect to CNS, decrease appetite and have other properties such like diuretic.	Piperine, conine, pilocarpine, trigonelline, nicotine, sparteine  Nicotine
2	Tropine	This group is characterized by	Atropine, pelletierine,

		containing the tropine nucleus	cocaine  Cocaine
3	Quinoline	This group is derived from tryptophan and strychnos which developed in nucleus	Quinine, strychnine, brucine, cevadine, dopamine, veratrine  Dopamine
4	Isoquinoline	This group are derived from tyrosine and phenylalanine	Opium alkaloids: papaverine, morphine, codeine, thebaine  Morphine ,
5	Indole-alkaloids	This group are synthesized from tryptophan. This part of group known to have hallucinogenic effect on CNS, cytostatic and antileukemic.	Tryptamine, reserpine, serotonin  Tryptamine

Alkaloids commonly found in the plant family such as Chenopodiaceae, Lauraceae, Menispermaceae, Berberidaceae, Leguminosae, Ranunculaceae, Papaveraceae, Papilionaceae, Fumariaceae, Apocynaceae, Rutaceae, Loganiaceae, Rubiaceae, Convolvulaceae, Boraginaceae, Campanulaceae, Solanaceae, Compositae, etc. [30].

Before doing test for the alkaloids, care must be taken because the reagent also may give precipitation reaction with the proteins. Hence, the extraction should be taken before doing the alkaloids test. Extraction will make the solution became protein free [4].

2.4.1.1. Extraction of Alkaloid

Extraction of alkaloids were performed based on their basic character and solubility profiles. Based on [30], alkaloids can be extracted with these two following method:

a. **Method A**:

Plant powder which containing alkaloid salts is moistened with alkaline substance (e.g. sodium bicarbonate, ammonia, calcium hydroxide, etc.) in combination with acids or tannin. During the extraction, alkaloid bases will be free then extracted with organic solvent. When the concentrate is shaken with diluted acid, the alkaloid salts will be extracted into the aqueous liquid.

b. **Method B:**

Extraction is done by adding the plant powder with alcohol containing diluted acid followed by adding the organic solvent or chloroform to remove the pigments and other unwanted material. Addition of alkali will precipitate the alkaloid and alkaloid can be further separated by extraction or filtration.

Extraction of volatile liquid alkaloids can be done by distillation. Plant powder that contains alkaloids is extracted using water followed by adding sodium carbonate or ammonia to make them alkali. The alkaloid is distilled off in the steam.

2.4.1.2. Chemical Test for Alkaloid Detection

Generally, most alkaloids are precipitated with solution containing metal or acid. Amorphous or crystalline precipitate may have various color depend on the reagent given[4]. There are several chemical tests to screening the presence of alkaloid based on [30]:

1. Dragendorff's reagent test

   Addition of dragendorff's reagent which contain potassium bismuth iodide will make alkaloids are precipitated to orange-reddish color

2. Mayer's reagent test

   Addition of mayer's reagent which contain potassium mercuric iodide will make creamy-white precipitation of alkaloids.

3. Hager's reagent test

   Addition of Hager's reagent which contain saturated aqueous solution of picric acid will produce crystalline yellow precipitate of alkaloid.

4. Wagner's reagent test

   Addition of wagner's reagent which contain dilute iodine solution will produce reddish-brown precipitate of alkaloids.

5. Tannic acid test

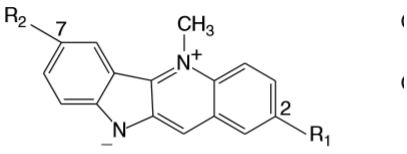
   Addition of tannic acid solution will form buff colored precipitated.

Yellow color will be shown when cochicine is treated with mineral acids, or bluish-violet will be turn into red if indole alkaloid is treated by sulphuric acid or *p*-dimethylamino-benzaldehyde [4].

2.4.1.3. Antimalarial Activity of Alkaloid

Beside of Quinine, there is an alkaloid compound named cryptolepine which was reported to has antimalarial activity. Starting from traditional knowledge in the usage of root's decoction of a climbing plant *Cryptolepis sanguinolenta* (Asclepiadaceae) as herbal treatment for malaria and other infectious disease in West

Africa, the indol-quinoline alkaloid named cryptolepine which is the major alkaloid was isolated and showed potent against *P. falciparum* but unfortunately showed toxicity. Cytotoxicity of this compound may be occurred because of this compound can intercalate into DNA, inhibit DNA synthesis and topoisomerase II. Toxicity in mice was shown during intraperitonial injection however not showed by oral administration. Less activity also was shown when doing the in vivo study. This may be happened because of this compound will be turn into inactive compound during metabolism and is absorbed slowly. The study showed that this compound will be metabolized in the liver and turn into inactive form, cryptolepine 11-one. Derivatization has been done to increase the activity and able to make the compound have no interaction with DNA. The 2,7-dibromocryptolepine is one product of derivatization process which is active by intrapertonial injection in mice without causing toxicity. Cryptolepine and its derivatives are known to have ability to inhibit β-haematin formation [4].



R_1, R_2 = H, cryptolepine (5)
R_1, R_2 = Br, 2, 7-dibromocryptolepine (6)

Figure 10. Structure of cryptolepine

[4]

2.4.2. Phenolic Compounds

Phenolic compounds are aromatic compound which have one or more hydroxyl group (-OH) connected to the aromatic ring. Phenols are crystalline solid molecule which have a scent, water soluble and have low melting temperature whereas the boiling point is high [32]. These compounds are secondary metabolites which derived from shikimate/phenylpropanoid pathway. Naturally, phenols play a role in defense mechanism from environmental pressure such as drought, cold,

microbial infection, predator, deficiency of nutrient. This condition can lead production of free radical [33]. Phenolics compounds are ubiquitous compound and can easily found in all organ of the plant. Generally, phenols are classified to flavonoids compound and nonflavonoids compound hence the flavonoids are the most bioactive and abundant rather than other groups. Flavonoids composed by phenyl benzopyran skeleton with two phenyl rings (A&B) joined through a heterocyclic pyran ring (ring C) [34].

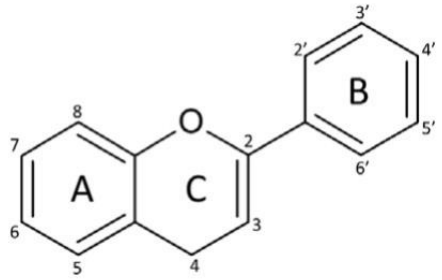


Figure 11. Basic structure of flavonoids

[34]

2.4.2.1. Main Type of Phenolic Compounds and Detection

Phenolic compound can be divided into several group based on the properties and complexity of the structure. These are the main type of phenolic compound:

A. Coumarin

Coumarin will give blue, blue-green, or violet fluorescence in ammonia solution [4]. These are several tests which commonly used for coumarin detection according to [30]:

Ferric chloride reagent test

Addition with $FeCl_3$ solution followed by adding the conc. HNO_3 will form green color turn to turned yellow color.

Fluorescence test

Addition with 1 ml of 1 N NaOH solution will form blue green fluorescence.

B. Anthraquinones

Usually these compounds are orange-red color and sometimes may observed direct in situ (*e.g.* in the cascara or modullary rays of rhubarb). This molecule can be dissolved in hot water or alcohol. Anthraquinones composed by free carboxylic acid group which can be breakdown by addition of mixture of organic solution and sodium bicarbonate [4]. According to [30], anthraquinone can be detected using these tests:

Borntrager's test

1 gram of plant material is added with dilute HCl followed by boiling and filtration. Filtrate extraction is done using organic solvent with addition of ammonia. After shaking, pink or red color will be present in ammonical layer.

Modified borntrager's test

1 gram of plant material is added with dilute HCl and 5% ferric chloride and boil. Filtrate is extracted with organic solvent and followed by adding ammonia solution. Pink to red color will be presence if there is C-type of anthraquinone.

C. Flavonoids

Flavonoids is known to be the largest groups of phenolic compounds which are over than 2000 compounds have been discovered with approximately 500 are the free compounds. Flavonoids can be dissolved in alkalis and when treating with alkali will turn into yellow color [4]. According to [30] flavonoids can be detected using these various methods of chemical test:

Ammonia test

When dipping filter paper in the sample solution is treated with ammonia vapor, yellow spot will show up.

Shinoda test

Addition of magnesium and diluted HCl will produce red color formation.

Vanillin HCl test

Addition of Vanilin HCl will form pink color.

D. Tannin

Tannins are complex phenol which non-nitrogenous, and usually have astringent property. The term ‘tannin” was first used in 1796 by Seguin because of its ability to react with animal hide to produce leather. This phenomenon became the basic of chemical reaction in detection using Goldbeater’s test. This test able to detect a true tannin while pseudo-tannin could not be detected. Tannin can be classified according to the hydrolysis capability [30].

Tannins are known to be rich in Geraniaceae, Combretaceae, Theaceae, Rubiaceae, Rosaceae, Polygonaceae, Leguminosae, etc. On the other hand, Papaveraceae and Cruciferae are totally devoid of tannins [30]. Tannin are oligomeric compound which have high molecular weight 500 - > 2000, crystalline, have characteristics like colloidal solution with water, not able to dissolved in alcohol and organic solvent except acetone, disolved in glycerin, alkali, water (except high molecular tannin), sparingly soluble in ethyl acetate, and have ability to bind with protein [30].

Tannin is derived from shikimic acid pathway (phenylpropanoid pathway) like other phenolics such as isoflavone, coumarine, lignins, and aromatic amino acid [30]. Tannins widely occurred in plants, commonly found in dead and dying cells. They have ability to inhibit various enzymes due to the precipitation reaction with protein hence became the protection properties of heartwood and bark. Tannin have ability to precipitate the alkaloid hence make the extraction is complicated and may produce the incompatibility. It had been known as antidotes by alkaloids heavy metals and glycosides poisoning [4].

Hydrolysable tannin

Hydrolysable tannins are composed by phenolic acids such like hexahydroxydiphenic acids and gallic acid which connected by ester linkages to the glucose chain [4]. This compound can be hydrolyzed by *tannase* or mineral acids. Based on hydrolysis of phenolic acids, hydrolysable tannin can be divided into gallotannin

(composed of gallic acid) and ellagitannin (composed of hexahydrodiphenic acid). They are soluble in water and while treated with ferric chloride will form blue color [30].

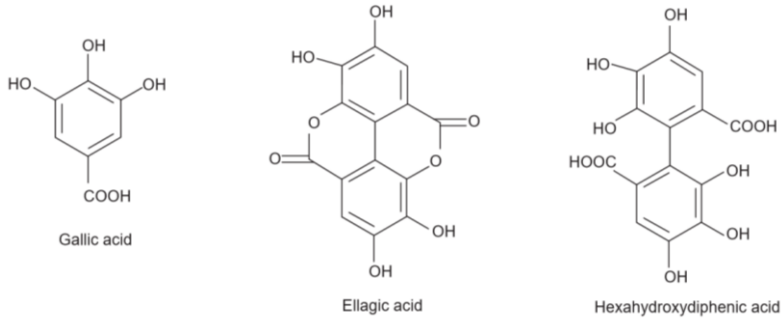


Figure 12. Structure of gallic acid, ellagic acid and hexahydroxydiphenic acid [30]

Nonhydrolysable or Condensed Tannins or Proanthocyanidins

Nonhydrolyzable tannins are molecules which could not be readily hydrolyzed by enzyme or mineral acids. Condensed tannin does not contain a sugar moiety [4] [30]. When condensed tannin is treated with acid, they turn into phlobaphenes, the red insoluble compound. Many drugs give red color by phlobaphenes (e.g. cinchona bark) [4].

Complex tannins

The complex tannins are derived from condensed tannin and hydrolysable tannin. However, this compound, is known to has less attention in pharmacognosy [4].

Chemical Test for Tannins

Tannin solution are precipitated when react with alkaloid, gelatin, heavy metals, and glycosides. Ellagitannins and gallitannins will show blue-black precipitates when treated with ferric salts whereas the condensed tannins give brownish-green color [4]. According to [30], these are several methods to detect the presence of tannins:

Goldbeater's skin test

Goldbeater's skin has properties like untanned animal hide which is produced from Ox's intestine. The sample solution is placed into the washed piece of goldbeater's skin which already soaked in 2% HCl. After washing, the piece of skin the dipped into solution of 1% ferrous sulphate. Tannin is detected when the color change to black or brown. This test may be worked in hydrolysable and condensed tannins while pseudo-tannins usually give negative or less color.

Phenazone Test

Addition of 0.5 g of sodium acid phosphate followed by warming, filtration, and addition with 2 % phenazone into the filtrate will make tannin precipitate as bulky color.

Gelatin Test

1% of gelatin solution is added with a few of 10% sodium chloride. Tannin will cause precipitation of gelatin.

Vanillin-hydrochloric acid test

Plant material will show pink or red color by addition of the mixture of 1:10:10 vanillin: alcohol: dilute HCl because of the production of phloroglucinol.

2.4.2.2. Antimalarial Activity of Phenolic Compounds

Phenolic compounds also have been known to has antimalarial activity (e.g. phloridzin, exiguaflavones, artemetin, casticin). Phloridzin, a flavonoid glycoside which has bitter taste like quinine is active inhibit malarial parasite. The compound able to inhibit the permeability of the membrane of infected red blood cell which lead deficiency of nutrient sources. Unfortunately, this compound is not suitable for application in clinical used because of it also have capacity to blocks the glucose reabsorption in the kidney. Additionally, exiguaflavone, a flavonoid which was isolated from *Artemisia indica* (Asteraceae) has been reported to active against *P. falciparum* while the artemetin and casticin, which were isolated from *Artemisia annua* also showed to act synergistically with artemisinin [4].

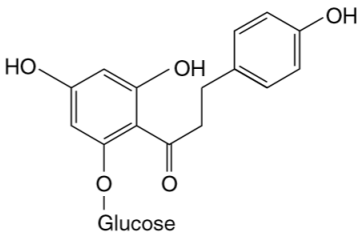


Figure 13. Structure of flavonoid glycoside (phloridzin)

[4]

2.4.3. Terpenes

Terpenoids are volatile compound which attribute to give the plant scent composed by hydrocarbons with the general formula $(C_5H_8)_n$. The compound may be hydrogenated, dehydrogenated or oxygenated. Isoprene is known to be basic unit of the terpenoid [30]. Based on [30], classification of terpenoids can be done based on the number of atom carbon:

Table 2. Terpenoid classes

Carbon atoms number	*n*	Molecular formula	Terpenoids Class
10	2	$C_{10}H_{16}$	Monoterpenoids
15	3	$C_{15}H_{24}$	Sesquiterpenoids
20	4	$C_{20}H_{32}$	Diterpenoids
25	5	$C_{25}H_{40}$	Sesterpenoids
30	6	$C_{30}H_{40}$	Triterpenoids
40	8	$C_{40}H_{64}$	Tetraterpenoids
>40	>8	$(C_5H_8)_n$	Polyterpenoids

2.4.3.1. Chemical Test for Terpenes Detection

These are several tests for detecting the terpenes according to [30]:

Libermann burchard test

Chloroform is used for terpenoid extraction. Addition of acetic anhydride followed by conc. H_2SO_4 will form violet to blue colored ring at the interphase of the two liquid. This indicate the presence of steroid moiety.

Salkowaski test

Chloroform is used for extraction. Addition of conc. H_2SO_4 will form yellow colored ring at the interphase of two liquid, which will be changed to red after 2 min. This indicate the presence of steroid moiety.

Antimony trichloride test

Chloroform is used for extraction. Addition of saturated solution of $SbCl_3$ in chloroform which contain 20% acetic anhydride will be produce pink color during the heating. This indicate the presence of triterpenoids and steroid moiety.

Trichloro acetic acid test

Addition of saturated trichloro acetic acid solution will form colored precipitation.

Tetranitro methane test

Addition of tetranitromethane will form yellow color in indication of unsaturated steroids and triterpenes.

Zimmermann test

Addition of meta-dinitrobenzene solution into extract in alkali followed by heating will form violet color in the keto steroid presence.

Terpenes (especially mono-, sesqui- and their oxygenated derivatives) are commonly found in volatile oils which are odorous and can easily to evaporate in room temperature. Volatile oils also known as essential oils because they represent essence of active constituent of the plant. Volatile oils usually produced in secretory cells but in few case, volatile oils are not preexisted and will be presented after glycosidae degradation (e.g. black mustard seed). Essential oils are insoluble water, soluble in non-polar solvent and fairly soluble in alcohol [30].

2.4.3.2. Antimalarial Activity from Terpenes

There are several compounds from terpenes group despite of artemisinin which had been studied in its antimalarial activity such as Yingzhaosu A and Brusatol. Yingzhaosu A is a sesquiterpenes which containing endoperoxide which is isolated from Chinese species Ying Zhao, *Artrobotrys unciatus* (family Annonaceae). This compound was known to be active against *P. berghei* in mice but less active than artemisinin. In order to improve the activity, its derivative Arteflene, has been developed and has evaluated in clinical trials but then abandoned because of the occurrence of recrudescence is high [4].

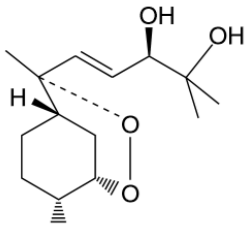


Figure 14. Structure of yingzhaosu A [4]

In other hand, brusatol is quassinoids which are derived biosynthetically from triterpenoid precusors. Brusatol was isolated from of *Brucea javanica* have been very active against *P. falciparum*. However, this compound is very toxic and the effort to improve its selectivity was not successful because both activities, the antiprotozoal and cytotoxic activities have acted in similar way which were inhibit the protein synthesis [4].

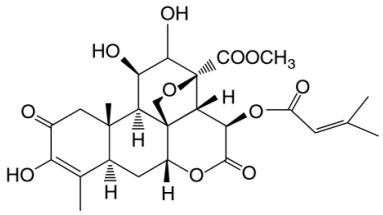


Figure 15. Structure of Brusatol

[4]

2.5. SESQUITERPENE LACTONES

Sesquiterpene lactones (SLs) are terpenoids compound composed by three isoprene unites connected to the cyclic esters, lactone group. The ϒ-lactone ring may contain hydroxyls, esterified hydroxyls or epoxide groups [15, 35]. The classification of SLs is based on their carbocyclic skeleton hence can be divide to germacranolides which have ten rings, eudesmanolides, eremophinalolides and guaianolides, pseudoguainolides and hypocretenolides. Many members of these group bear open ring structure [36].

Sesquesterpene lactones is secondary metabollite which is typically located in laticifers (secretary cell in most Asteraceae) but can be found in vacuoles of other cell types especially during the respons to biotic stress [37]. Sesquesterpene lactone (SLs) is characterized by its bitter, colourless substance, and liphophilic. They commonly found in Asteraceae family plant [15] but can also found in Magnoliacea, Lauraceae and Apiaceae [38]. These compounds play role as antifeedants, deterrents, attractants, communication between plants and others organism. Each species usually produces one specific type of SLs [35].

SLs can be extracted by liphopilic solvent or liquid (supercritical) carbondioxide. It can be isolated from all plant organs but commonly in leaves and glandular trichome of the leaves [38]. Solvent commonly used for extraction this compound including petroleum ether, n-hexane, acetonitril, chloroform, methanol, toluene and their combination [15].

Based on the research, several sesquesterpene lactones from *Distepanus angulifolius* show antimalarial activity against *P. falciparum* [38, 39]. 2 sesquesterpene

lactones from *Cyperus articulatus* have significant antiplasmodial properties [40]. Pseudoguainolide sesquesterpene lactones have high activity againsnt *Plasmodium falciparum* [41].

2.6. NATURAL PRODUCT EXTRACTION

Extraction method can be chosen according to the character of the plant material and the compound which will be extracted. Extraction target can be a known or unknown bioactive compound, structurally related group of compounds, specific secondary metabolites or all present secondary metabolites [42]. Extraction step is performed after selecting, collecting and authenticating the plant. Selection of interested plant can be conducted based on history of traditional use, random selection, toxicity, chemical content, or combination of them. Plant collection should be involved a botanist whose have ability to identify specimen correctly. Herbarium specimen should be prepared with the record of the place and collection date [43].

After plant collection, the next step is stabilization. Drying is one of the most usual method to stabilize the material and can be done under ambient temperature or using heating oven. Other stabilization method can be conducted such like lyophilization, freezing, alcohol vapour, etc [43].

Based on [42], dry condition is essential to prevent microbial and degradation of the metabolites (e.g. for the thermolabile and light-sensitive compound, protection from high temperature and direct sunlight should be performed to minimize chemical reaction). Recommended temperature for drying is under 30 ^{O}C. However, for the country with high humidity, oven can be used to accelerate the drying process. The remaining moisture and water in plant due to the long-time drying process may stimulate the enzymatic reaction (e.g. hydrolysis of glycosides). Based on [44], drying temperatures in between 50 °C to 60°C is apparently feasible for applying in mostly plants.

Drying method may affect the composition of chemical compounds. Comparation in chemical composition of essential oil of basil (*Ocimum basilicum* L.) submitted to drying with air heated to 45°C and with those obtained from fresh plant

(control). The composition of essential oil of dried basil showed a chromatographic standard very different from that obtained in control. The contents of methyl chavicol and eugenol decreased during drying, however, the levels of trans-bergamotene, linalool and 1,8-cineole significantly increased. The effect of sun-drying, shade-drying and oven-drying at 45°C in chemical composition of *Juniperus phoenicea* L. essential oils also have been studied. The authors concluded that drying of berries of *J. phoenicea* in oven-drying was more suitable and was recommended for obtaining higher yield of essential oils (for higher percentages of some special components). However, such as α-pinene and δ-3-carene, the result showed that shade-drying was more suitable [44].

After drying, dried material can be stored in dry condition up to 6 months. Dried powder can be obtained through grinding process. Grinding is functioned to improve the wide of contact surface area between solvent and plant material. Based on [45], generally, finer particles will accelerate the extraction efficiency which can enhance the penetration of extraction solvents. If the targeted compound is known, the extraction method can be directed targeting the compounds. On other hand, if the compound is unknown, the extraction procedure can be done due to the traditional uses or using several solvents with different polarity [43].

2.6.1. Solvent Selection

The selection of the solvent should be considered in case of the solubility, selectivity, safety and the cost. Principle of extraction is “like dissolve like” which mean that polar solvent will extract the quite polar molecule while non-polar solvent will extract the nonpolar molecule. Polarity of the common solvent can be shown in this following table:

Table 3. Polarity index of the solvents [46]

Solvent Extraction	Polarity Index	Boiling points (^{0}C)	Density (25^{0}C g/mL)
Water	10.2	100.0	1.000
Ethanol	5.2	78.0	0.789
Acetone	5.1	56.0	0.791

Methanol	5.1	64.7	0.792
Chloroform	4.1	60.5-61.5	1.492
Isopropanol	3.9	82.0	0.785
Dichloromethane	3.1	39.8-40	1.325
Diethyl ether	2.8	34.6	0.706
Benzene	2.7	80.0	0.874
Toluene	2.4	110.0-111.0	0.865
Isooctane	0.4	99.2	0.690
Cyclohexane	0.2	80.7	0.779
Petroleum ether	0.1	25.0-60.0	0.640
Hexane	0.1	69.0	0.659

Table 4. Solvent for phytochemicals extraction [47]

Water	Ethanol	Methanol	Ether	Acetone	Chloroform
Anthocyanins	Alkaloids	Anthocyanins	Alkaloids	Flavonols	Flavonoids
Lectins	Flavonols	Flavones	Coumarins	Phenol	Terpenoids
Polypeptides	Polyacetylenes	Lactones	Fatty acids		
Saponins	Polyphenols	Phenones	Terpenoids		
Starches	Sterols	Polyphenols			
Tannins	Tannins	Quassinoids			
Terpenoids	Terpenoids	Saponins			
		Tannins			
		Terpenoids			
		Totarol			
		Xanthoxyllines			

The solvent will selectively extract some compounds based on the polarity. However, some solvent used as universal solvent for “total extraction” due to the structure of the molecule which may bearing both hydrophilic and lipophilic (amphiphilic

property) hence may extract the compounds which have low polarity until high polarity (e.g. ethanol, methanol and water) [42].

During manufacturing the drug, the solvent which cannot been removed completely from the drug defined as residual solvent. Because these residual solvents are having no therapeutic benefit and most of them have toxicity and should be limited in the intake, so the residual solvent must be removed. Based on ICH (International Council on Harmonization of Technical Requirement for Registration of Pharmaceutical for Human Use) guideline 2016, the residual solvent can be classified into 3 group class based on the safety and toxicity.

Table 5. Residual solvent class classification [48]

Residual Solvent	Attention
1st Class	Should be avoided, carcinogen to human, enviromental hazard
2nd Class	Should be limited, nongenotoxic animal carcinogens, cause reversible or irreversible toxicity (e.g. as neurotoxic or tetragonicity)
3rd Class	low toxicity, there is no health-based limit is needed (PDEs of 50 mg or more per day)

Table 6. 1st class solvents [48]

Type of solvent	Limit (ppm)	Attention
1,1,1-trichloroethane	1500	Environmental hazard
1,1-dichloroethene	8	Toxic
1,2-dichloroethane	5	Toxic
Carbon tetrachloride	4	Toxic and environmental hazard
Benzene	2	Carcinogen

Table 7. 2nd class solvent [48]

Type of solvent	PDE (mg/day)	Limit (ppm)
Methylisobutylketone	45	4500
Cyclohexane	38.8	3880
Methanol	30.0	3000
Xylene*	21.7	2170
1,2-Dichloroethene	18.7	1870
Methylcyclohexane	11.8	1180
N,N-dimethylacetamide	10.9	1090
Toluene	8.9	890
N,N-dimethylformamide	8.8	880
Tetrahydrofurane	7.2	720
Ethyleneglycol	6.2	620
Dichloromethane	6.0	600
N-methylpyrrolidone	5.3	530
Acetonitrile	4.1	410
1,4-dioxane	3.8	380
Chlorobenzene	3.6	360
Hexane	2.9	290
Formamide	2.2	220
Pyridine	2.0	200
2-ethoxethanol	1.6	160
Sulfolane	1.6	160
1,2-dimethoxyethane	1.0	100
Tetralin	1.0	100
1,1,2-trichloroethene	0.8	80
Cumene	0.7	70
Chloroform	0.6	60
2-methoxyethanol	0.5	50
Methylbutyl ketone	0.5	50
Nitromethane	0.5	50

Table 8. 3rd class solvent (less toxicity) [48]

Tert-butylmethyl ether	2-methyl-1-propanol
Formic acid	Triethylamine
Ethyl formate	Propyl acetate
Ethyl ether	2-propanol
Ethyl acetate	1-propanol
Ethanol	1-pentanol
Dimethyl sulfoxide	Pentane
Butyl acetate	Methylethyl ketone
Anisole	Isopropyl acetate
Acetone	Isobutyl acetate
Acetic acid	Heptane
2-butanol	3-methyl-1-butanol
1-butanol	Methyl acetate

2.6.2. Extraction Techniques

There are several methods of extraction for plant natural product commonly used:

2.6.2.1. Maceration

Maceration is one of the most common method in the extraction technique. The technique is quite easy, convenience, no need much equipment and skill. The principle is the plant material is keep soaked into solvent for a period of the time. This method is suitable for a thermosensitive substance. However, large volume of solvent is needed, can be time consuming and has a low extraction efficiency [45, 49]

2.6.2.2. Soxhlet extraction

Soxhlet extraction is continuous hot extraction which can be applied in initial or large extraction. The powdered plant is put into thimble of soxhlet which is connected to the flask containing solvent. The solvent is continuously heat up under reflux and will begin to be evaporated, cooled with the condenser then extract the plant material [42]. This technique is required when targeted

compound is less or limited soluble in the solvent to enhance the extraction rate. This method only require a small volume of the solvent because the solvent is being recycle and reused again but this method isn't suitable to be used for thermolabile/heat sensitive compound which may lead degradation of compound during the heat [47]. This degradation is reported when extracted catechins in the tea. There is other report which demonstrated that total polyphenols and alkaloids during soxhlet extraction at 70 °C is decreased compared to maceration at 40 °C [45].

2.6.2.3. Decoction

Decoction method have same principle as maceration but using boiled water to extract the plant material [47]. Decoction is not suitable for extracting the heat-sensitive compound, this method is usually suitable for extraction of hard plants parts like roots and bark. Usually, extraction product is more oil soluble compared to infusion and maceration [45].

2.6.2.4. Infusion

This method also has same principle as maceration but using hot or cold water as a solvent. The period of infusion also shorter than maceration.

2.6.2.5. Digestion

The principle is maceration with applying warm temperature (40-60 ^{0}C) during extraction process [50].

2.6.2.6. Percolation

This method is the most common procedure to extract the active ingredient from fluid extracts by using percolator to extract the compounds [50]. Powdered plant is place in the percolator followed by pouring the solvent on the top material and keep allowed to extract slowly. Additional filtration is not needed because the filter already set in in the percolator. This technique is suitable for small and large extraction and can be performed exhaustively [42]. However, the material which easily swelling such like mucilages or resins could clog the percolator. Furthermore, material which not distributed homogenously will make the extraction may not completely done. Higher temperature will

enhance the rate of extraction but may lead the degradation of thermolabile compound. This process required a large volume of solvent and can be time consuming [42].

2.6.2.7. Ultrasound assisted solvent extraction/ sonication

This technique is maceration which enhanced by ultrasound with high frequency. Plant material is soaked into the chamber which already fill in with the solvent followed by placing the chamber in ultrasonic bath. The pulse of ultrasound frequency (20-2000 kHz) will induce the cavitation and mechanical disruption of the cells which can enhance the solubilization of the solvent to extract the metabolites. Frequency, length of extraction and temperature will affect the extraction efficiency. This extraction is rarely used in bulk extraction but mostly used in small amount material for initial extraction [42] [47]. However, ultrasound energy which more than 20 kHz will induce production of free radicals and unwanted changes of metabolites [47]. The strength of this technique is convenience to extract the thermolabile compound, less time and solvent consuming [45].

2.6.2.8. Pressurized solvent extraction (accelerated solvent extraction)

This method used higher temperature than other method and need maintain that liquid state of solvent in high temperature using the pressure. This temperature and pressure will increase the rate of extraction. Rapid and reproducible extraction can be obtained using this method. Materials is placed into the extraction cell in the oven. The cell is filled with the solvent which is heat up and pressurized for period of the time. Nitrogen gases will be flushed into the cell to concentrate the extract and filtrate will be collected automatically. The remaining extract can be collected by rinsing the cell with new solvent. This method is not solvent consuming and reproducible. However, optimization in the temperature, time, kind of solvent should be done first to obtain the good yield in the faster period [42].

2.6.2.9. Reflux and steam distillation

Plant material which is soaked in the round bottle which already fill in with the solvent is connected to a condenser. The solvent will be heat until obtain its boiling point. Solvent will be evaporated and condensed and recycle to the system. This method is commonly used for to essential oils extraction. During this process, the oils will be collected when aqueous solution is recirculated into system. This method is suitable for thermo-stable compound [42].

2.6.2.10. Microwave Assisted Extraction (MAE)

This technique is called a "green extraction" because the method uses the less energy and solvent. The electromagnetic waves from the microwave will selectively vapour and heat the polar molecules. This method can be solvent free or not. Additionally, this method can be applied for extraction such a heat-labile compound using an appropriate solvent. However, MAE may limit only to small molecule which stable under microwave (e.g. gallic acid, ellagic acid, isoflavin, quercertin, and trans-resveratrol). The study reported that additional cycles from 2×10 s into 3×10 s was decreasing the phenolics content. It may be happened because of oxidation. Tannins or anthocyanins also mentioned to be not suitable to apply by this method because of the possibility of degradation [45].

2.7. ANTIMALARIAL SCREENING ASSAY

There are several assays to screening the antimalarial activity such as schizont maturation inhibition assay, the titrated [^{3}H]-hypoxanthine incorporation, lactate dehydrogenase (LDH) assay and SYBR green assay [4, 51]. The strain *P. falciparum* which have been commonly uses are chloroquine-sensitive (CQS) (e.g. , NF54, 3D7, D10, D6, RKL2, TM4) and chloroquine-resistant (CQR) (e.g. FCR3, INDO, FcM29, FCB, W2, K1, K1CB1 and multi drug-resistant parasites strain [51]. Based on [7], these are the several test for antimalarial activity screening:

2.7.1. Microtest

Microtest has developed by Rieckmann et al (1978) and this technique then adopted under the sponsorship of WHO to design a field study. This technique is designed for laboratory tools to do the surveillance in the part of global monitoring program Microtest is defined as counting the schizont using simple microscopy. Thick blood smear is prepared then the total number of schizonts are counted against 500 leukocytes. *In vitro* activity is determined by calculate the percentage of counted schizonts in the treatment compared to control.

2.7.2. Radioisotopes based assay

This technique using radioisotopes such as [^{3}H]-hypoxanthine, [^{3}H]-ethanolamine radioisotopes to measure the parasites. This radioisotope is widely used by people this compound is main purine which is needed by the parasites. Radioisotope incorporated is define as parasites count. This technique needs the parasitemia between 0,1% and 1% at 1,5% haematocrit during 42h of incubation. Whereas [^{3}H]- ethanolamine is known to be more incorporated with the infected erythrocytes compared to [^{3}H]-hypoxanthine.

2.7.3. Enzyme based assay

This technique is to check the parasite viability by measuring the parasite lactate dehydrogenase (pLDH) which have vital role in glycolytic in anaerobic metabolism. This assay is functioned to monitor the ability of LDH enzyme using APAD (NAD analog) which able to convert lactate into pyruvate. Reducing APAD will be measured represent correlation between pLDH activity and parasitemia. Because of the measurement need high parasite density (1-2) it was difficult to apply in the field examination. Hence to overcome those problem, DELI assay was developed. This technique based on monoclonal antibody specific for pLDH. This technique able to detect very low level of parasitemia (< 0.005).

2.7.4. Flowcytometry Assay

Basic principle of this technique is human erythrocytes lack DNA so the stain can special detect the parasite DNA. The whole parasite will be stained hydroethidine or fluorescence DAPI. This technique able to differentiate different blood stage of parasites hence the schizont maturation enable to determine while also counting the nuclei parasite so the exact number of parasites can be obtained. Strengthness of this technique is its ability to count the level of parasitemia accurately and also can differentiate the developmental stage of the parasites.

2.7.5. Fluorescence Based Assay

This technique uses DNA binding dye (e.g. ethidium bromide) or DNA intercalating dye (e.g. SYBR Green I, Pico Green and YOYO-I). DNA and RNA are not present in the erythrocytes so the dye will specifically bind to DNA of the parasite. The DNA intercalating dye is safer than ethidium bromide because have less mutagenicity. The advantage of this assay is less time consumption, both of unsynchronized and synchronized parasites can be measure with no significance difference, faster (only take time 48 -72 h) and no need special skill.

2.7.6. *In vitro* beta-hematin Formation Assay

Hemozoin or beta-hematin is non-toxic metabolic product which released during heme metabolism. Principle of this method is to measure the hemozoin formation to indicate the living parasite in the RBCs. Measurement can be done by various technique such as spectrophotometric, radioisotopic, fluorometric, HPLC, FT-IR spectroscopy and the result can be interpreted after 12-24 h of incubation with treatment.

2.7.7. *In vitro* Assay Targeting Liver Stages

HepG2/primary hepatocytes incubate for about 24h followed by inoculation of the sporozoites. After incubation for 48h quantification can be done using combination of infrared imaging system and colony counter.

2.8. GENETIC DIVERSITY

DNA barcoding can be done using various technique including use of the sequences of internal transcribed spacer (ITS). Despite ITS, there are common nuclear sequences which being used for plant DNA barcoding such as intergenic spacer (IGS) and plastid genes (*rbcL, matK, psbA-trnH,* intron, *trnL*, etc). The ribosomal DNA (rDNA) ITS region shows relatively high authentication of efficiency, mutation and evolution rates regarding to the length and sequence [12]. Its high resolution of inter- and intraspecific relationships made this region widely used for plant molecular systematics at the generic and species levels [13]. This region can be amplified universally using the primer in the conserved region of the rDNA repeat (18S and 26S genes) [14].

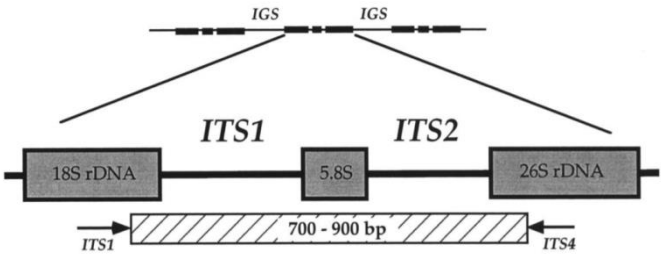


Figure 16. Structure of the internal transcriber spacer (ITS) region [11]

Ribosomal DNA genes occur as arrays of tandem repeats which dispersed in various location in the genome. This repeating units include 26S rDNA, ITS2, 5,8S, ITS1 and 18S rDNA gene which are separated by non-transcribed intergenic spacers (IGS) [11].

Phylogenic tree represent relationship between species which is showed in the diagram called cladogram. In the phylogenetic tree, we can know the clade of mono-phyletic, para-phyletic and poly-phyletic. Monophyletic group is defined as the all group of species are shared and derived from the common ancestor. Paraphyletic group is defined as the group of species share common ancestor but there are species which isn't derived from that common ancestor. On other hand, polyphyletic group is mean that the

group of species aren't shared common ancestor [52]. Phylogenetic tree can be constructed using free website.

Table 9 Different method for phylogenetic analysis [52]

Method	Distance elimination	Phylogeny method	Available free software	Note
UPGMA (Unweighted pair-group method using arithmetic averages)	Yes	Clustering	MEGA, Phylip	Follow molecular clock hypothesis
NJ (Neighbor Joining)	Yes	Clustering	MEGA, Phylip	Minimum evolution
Fitch-Margoliash	Yes	Clustering	MEGA, Phylip	Minimum evolution
ME (Minimum Evolution)	Yes	Clustering	MEGA, Phylip	Minimum evolution
Maximum parsimony	No	Multiple trees	MEGA, Phylip	Parsimony hypothesis
Maximum likelihood	No	Multiple tree	MEGA, Phylip, PAML, HyPhy, PhyML, PUZZLE	Likelihood method
Bayesian	No	Multiple tree	MrBayes, BAMBEE	Likelihood hypothesis and is extension to the ML

2.9. DESCRIPTION SELECTED ASTERACEAE MEDICINAL PLANTS

Figure 17. Selected Asteraceae medicinal plants.

1. *Artemisia vulgaris,* 2. *Artemisia lactiflora,* 3. *Blumea balsamifera,* 4. *Ageratum conyzoides,* 5. *Bidens pilosa,* 6. *Vernonia cinereal,* 7. *Gynura divaricata,* 8. *Gynura pseudochina*, 9. *Tridax procumbens,* 10. *Wedelia trilobata,* 11. *Eupatorium odoratum,* 12. *Artemisia dracunculus,* 13. *Eupatorium capillifolium,* 14. *Artemisia chinensis,* 15. *Sphaeranthus indicus,* 16. *Acmella oleracea*

2.9.1. Artemisia vulgaris

Vernacular name:

Common name	Mugwort
Local name	โกฐจุฬาลัมพาไทย Khot Chulalampua Thai
Synonym	*Absinthium spicatum, Artemisia affinis, Artemisia apetala,*

	Artemisia cannabifolia, Artemisia coarctata, Artemisia discolor, Artemisia dubia, Artemisia eriophora, Artemisia flodmanii, Artemisia glabrata, Artemisia heribaudii, Artemisia heyneana, Artemisia hispanica, Artemisia indica, Artemisia javanica, Artemisia leptophylla, Artemisia leucophylla, Artemisia longiflora, Artemisia ludoviciana, Artemisia michauxii, Artemisia officinalis, Artemisia opulenta, Artemisia paniculiformis, Artemisia parviflora, Artemisia princeps, Artemisia quadripedalis, Artemisia rubriflora, Artemisia ruderalis, Artemsiia samamisica, Artemisia selengensis, Artemisia superba, Artemisia violacea, Artemisia virens, Artemisia wallichiana

Distribution:

This plant can be found in agriculture landscape, waste areas and on the road-side[53].

Morphology:

Rhizomatous perennial weed, erect stem, branched or unbranched, short or tall, can reach up to 2 m, striated or grooved deeply. Stems have based with green into brown colour while the upper is purplish and sometimes hairy. The stem can reach up to 0.4-1.5 m in height spreads rapidly, rooting by rhizome system, long stemmed 70-150 cm. The leaves are pinatissect or bi-pinatissetic, segment is oblong or lanceolate, soft and the colour in the dorsal part is white silver. Light brown rhizome which can reach up to 1 cm in diameter and able to reach in the depth in soil for 7–18 cm. Leaves are sessile, pinnate, dark green, 5–20 cm in long, there are white tomentose hairs on the leaves underside [53, 54].

Mugwort's flowers almost glabrous, red brown or yellowish color, ovoid flower head with 3–4 mm in long and 2 mm in wide. Inflorescence type clusters are discoid, pendulums numerous and small. Arrangement in racemes panicles with involucral oval bracts, obtuse, hairy receptacle, with hyaline edges, hermaphrodite, corolla tubular-

filiform, the hermaphrodite disc with tubuler corolla with five lacinios, fruit achene type, cylindrical or flattened, usually glabrous without papus or occasionally pubescent, fruit has indistinct margin [53]. Mugwort fruit has an indistinct margin [53].

Traditional Uses:

Roots, aerial part and stems has been used for various treatments [53]. In northern America latin, the juice of flowers, flower buds and leaves are being use for malaria treatment [55]. Based on [54], the infusion of the leaves is used to treat fever.

Antimalarial Activity:

The methanolic extract using reflux extraction of leaves of this plant can inhibit *P. falciparum* FCR-3. After treatment of methanolic extract of leaves at the concentration 6.5 µg/ml showed aparasites growth at 73% while the water extract at 7 µg/mL showed 78% parasites growth [56]. In other hand, ethanolic extract of aerial part of Iran herbs using lactate dehydrogenase assay method had shown the IC_{50} > 200 µg/mL against both of *P. falciparum* strain K1 and CY27 which can be categorized inactive [57, 58].

Phytochemical compound list:

The plant known to contained artemisinin but lower concentration than other *Artemisia* hence this plant has not been used for artemisinin source for commercial ways. However, this plant has been applied in various fields such as cosmetic, pharmacy and food. The major **flavonoid** content is eriodictyol and luteolin while also contain others flavonoids such as flavone glycosides (luteolin 7-glucoside and vitexin), flavones (tricine, chrysoeriol, jaceosidine, diosmetin, apigenin, eupafolin), flavonol glycosides (kaempferol 7-glucoside, kaempferol 3-glucoside, kaempferol 3-rhamnoside, kaempferol 3-rutinoside, quercetin 3-glucoside, quercetin 3-galactoside, quercetrin and rutin) and flavonols (isorhamnetin), flavanones (homoeriodictyol and eriodictyol). The whole plant's **volatile oils** contain α-thujone, camphor, α-pinen, 1,8-cineole, camphene, germacrene D, β-caryophyllene. Leaves contain kaempferol-3-rhamnoside, kaempferol-3-glucoside, apigenin, luteolin rutinoside, quercetin, quercetin 3,7-dimethyl ether,

quercetin 3,3′-dimethyl ether, quercetin 3-galactoside, quercetin-3-malonylglucoside, rutin, phenolic acid such as 5-O-feruloylquinic acid, 1,5-O-dicaffeoylquinic acid, organic acid such as quinic acid, malic acid, trihydroxy-octadecenoic acid, acid glucoside and tuberonic, **sesquesterpene** such as artemisinic acid glucoside, artemisinin, artemisinic acid, yomogin and 1,2,3,4-diepoxy-11(13) eudesmen-12,8-olide, lignan such as trachelosidea and **monoterpene** such as dehydrovomofoliol [53]. [59] report that this plant contains eudesmane-type sesquiterpene, morin, luteolin, **triterpenes**, coumarin, flavonoids, eriodictyol. According to the research report which has been conducted by [60], the amount of artemisinin in the leaves was the same while compared to the flowers. The concentration of artemisinin is lower than *A. dracunculus* and *A. annua.* Report from [61] showed that this herb contained camphor, borneol, p-cymene, fenchone, α-thujone, β- thujone, cineole, geraniol, β-pinene, 4-terpinenol, α-terpineol, sterol, caffeoylquinic acids, caryophyllene and coumarin.

Pharmacological activities:

This herb has various activities including larvicidal, antifungal, antibacterial, antioxidant, antitumor, antimicrobial, preservatives in cosmetics and pharmaceutical, antispasmodic, antiseptic, antimalarial, hepatoprotective and antirheumatic qualities [53] [62].

2.9.2. Artemisia lactiflora

Vernaculare name:

Common name	White mugwort
Thailand local name	จิงจูฉ่าย Jing ju chai
Synonym	*Artemisia septemlobata*

Morphology:

This herb is large clump forming non-invasive perennial plant, can reach up to 6 feet and width of 3-4 feet. Sterile flowers are white to creamy white. Leaves is toothed, deep green with silver undersides. This herb is slightly aromatic [63].

Distribution:

This herbs mainly distributed in Southeast Asia [64].

Traditional uses:

In Chaosan China, whole plant of this herbs is being used to heat clearing [65].

Antimalarial Activity:

There is no scientific research report about antimalarial activity.

Phytochemical list:

This herb contains 7-hydorxycoumarin, aurantiamide acetate, aurantiamide, caffeic acid, balanophonin, 7-methoxycoumarin, methyl 3,5-di-O-caffeoyl quinate, iso-vitexin, kaempferol-3-O-beta-D-rutinoside, quercetin and rutin. The leaves contain beta carotene, lactone, ascorbic acid, bitter absinthin, anabsinthin and riboflavin [66]. Aerial part contained polyacetylene, artemisidiyne [64].

Pharmacological activities:

Antioxidant, anti-cancer, controlling blood circulation, chronic hepatitis, dysmenorrhea, vaginal discharge and cirrhosis [66].

2.9.3. Blumea balsamifera

Verniculare name

Common name	Blumea champor
Local name	หนาดใหญ่ Nad yai
Synonym	*Baccharis balsamifera, Baccharis salvia, Baccharis gratissima, Blumea appendiculate, Blumea grandis, Blumea zolligeriana, Conyza appendiculate, Conyza balsamifera, Conyza saxatilis, Pluchea appendiculate, Pluchea balsamifera*

Distribution:

This herb is distributed in India to Sourthern China and throughout Southeast Asia [67].

Morphology:

Subshrub, perennial herb, can grow up to 1-3 m in tall. Strong, erect and taupe, stem which have longitudinal edge. Dense non glandular hair has covered their upper internodes. The leaves are oblong, lanceolate, ovoid, 22–25 cm in length and 8–10 cm, attenuated petiole in the base of the leaves, linear appendant is narrow (3–5 pairs in each side, pubescence, lateral vein 10–15 pair. The color is slightly brown or may silky-villous yellowish white. The flowers are yellow, have numerous female parts, receptacle honeycomb, corolla tubular and thin [68].

Traditional uses:

Malaysian used this herb to malaria treatment. The whole plant, leaves or the roots is used as anti-plasmodial. Leaves decoction is used for fever, influensa and coughs [69]. Vietnamese people also use this herb to treat malaria and fever [70].

Antimalarial Activity:

Ethanolic extract from root and stem from Malaysian herbs can inhibit *P. falciparum* sensitive strain D10 using LDH assay which showed IC_{50} root 26.25 ± 2,47 µg/ml; stem 7,75 ± 0,35 µg/ml and do not have cytotoxicity at MDBK cell [69]. Whereas report from Indonesia show that methanolic extract of *B. balsamifera* leaves show IC_{50} 8,7500±1,21 µg/ml against *P. falciparum* 3D7 using schizonticidal/giemsa blood smear assay [71].

Phytochemicals list:

Based on [68], the herbs contain **monoterpene** such as L-borneol, isoborneol, limonene, ocimene, α-terpineol, β-ocimene, β -myrcene, α-thujene , champene, β-pinene, α-pinene, terpinen-4-ol, chrysanthenone, perillyl alcohol, bornyl acetate, sabinene, linalool oxide and 1,8-cineole; **sesquesterpene**: α-gurjune, alloaromadendren, (+)-aromadendrene, aromadendrene, aromadendrene oxide, aromadendrene, dehydro, longifolene, α-caryophyllene, caryophyllene oxide, β-caryophyllene, guaia-3,9-diene, δ-cadinene, γ-cadinene, β-selinene, γ-gurjunene, β-gurjunene, thujopsene-13, β-elemene, 10-epi-γ-eudesmol, globulol, (−)guaiol, ledol, γ-muurolene, elemol, α-eudesmol, β-eudesmol, γ-eudesmol, cubenol and carotol; **diterpenes**: cryptomeridiol, 16-kaurene, 1-ang-4,7-dihydroxyeudesmane, phytol, blumeaene; **fatty acid**: trans-2-undecenoic acid, 9-hexadecenoic acid, capric acid, palmitic acid, (11 Z)-11-hexadecenoic acid; **phenol**: xanthoxylin, eugenol, dimethoxydurene; **flavone**: luteolin, luteolin-7-methyl-ether, diosmetin, chrysoeriol and 4',5-dihydroxy-7-methyletherflavanone ; **flavonols**: 3,5,3',4'-tetrahydroxy-7-methoxyflavone, quercetin, 3,5,3'-trihydroxy-7,4-dimethoxyflavone, rhamnetin (7-methoxyquercetin), tamarixetin, chrysosplenol C, ayanin, hyperoside, ombuine, isoquercitrin; **flavanones**: blumeatin (5,3',5'-trihydroxy-methoxydihydroflavone), eriodictyol, 5,7,3',5'-tetrahydroxyflavanone, 3',4',5-trihydroxy-7-metoxyflavanone; **flavanols** : catechin, (2R,3R)-(+)-7-O-methyldihydroquercetin; **coumarin**: dydranngetin, umberlliferone (7-hydroxycoumarin); **sesquesterpene lactone**: blumealactone.

Pharmacological Activities:

Hepatoprotective, antioxidant, anti-tumor, anti-microbial and anti-inflammation, anti-obesity, anti-tyrosinase, anti-plasmodial, platelet aggregation, wound healing, enhancing percutaneous penetration [68].

2.9.4. Ageratum conyzoides

Vernacular name:

Common name	Goat weed, billygoat weed, chicken weed
Local name	หญ้าสาบแร้ง Ya sap raeng/ ya sap haeng/ tap suea lek
Synonym	*Ageratum album, Ageratum arsenei, Ageratum brachystephanum, Ageratum ciliare, Ageratum coeruleum, Ageratum hirsutum, Ageratum hirtum, Ageratum humile, Ageratum iltisii, Ageratum latifolium, Ageratum microcarpym, Ageratum muticum, Ageratum nanum, Ageratum obtusifolium, Ageratum odoratum, Ageratum suffruticosum, Alomia microcarpa, Cacalia mentrasto, Caelestina latifolia, Caelestina microcarpa, Carelia brachystephana, Carelia conyzoides, Carelia mutica, Eupatorium coyzoides, Eupatorium palaeceum, Sparganophorus obtusifolius*

Morphology:

Annual, branched herb, fine white hair covered the entire stems and leaves, can grow up to 1 m in height. Leaves are ovate 7.5 cm in length. Terminal inflorescence, white or purple. Fruits are easily dispersed and achene. The odor is specific like male goat [72]

Traditional uses:

This herb has been used to treat fever in Asia, South America, Africa [73][55].

Antimalarial Activity:

The study report from S. Tome and Principle Island, Gul Guin has been shown that the ethanolic extract of aerial part of this herbs can inhibition the *P. falciparum* Dd2 with the giemsa blood smear schizonticidal assay with the IC50 median result 150 µg/ml [74]. Whereas report from Nepal, the ethanolic extract using soxhlet extraction was shown inhibition against *P. falciparum* CQ resistant 2/K1 using nitro blue tetrazolium assay with IC_{50} 72.4 ±28.3 µg/ml and the cytotoxicity of 62.7± 3.3 µg/ml [73].

Phytochemical list:

The oil content are **monoterpenes**: limonene, β-pinene, sabinene, β-phellandrene, 1,8-cineole, terpen-4-ol, α-terpineol; **sesquesterpene**: β-caryophyllene, δ-cadinene ; coumarin, chromenen, chromone, benzofuran; **sterol**: beta sitosterol, stigmasterol; **alkaloid**: lycopsamine, echinatine, sesamin, fumaric acid, caffeic acid, phyto, aurantiamide asetate. Whereas the leaves contain **sesquesterpene**: sesquiphellandrene, caryophyllene epoxide. Stem contained isoflavon glycosides [72].

The report study from [75] this herb contains flavonoid and chromene which have antiprotozoal activity. The flavonoids are ageconyflavone, eupalestin, 5'-methoxynobiletine, 5,6,7,3',4'5'-hexamethoxyflavone, kaempferol and catechin whereas the chromenes are precocene I, encecalol angelate.

Pharmalocogical Activities:

Anti-microbial, anti-consvultan, neuromuscular blocking activity, analgesic activity, anti-inflammatory, anti-cancer, anti-depressant, insecticidal activity [72].

2.9.5. Bidens pilosa

Vernaculare name:

Common name	Black-jack, beggar-ticks, cobbler's pegs
Local name	ปืนนกไส้ Puen noksai
Synonym	*Bidens abadiae, Bidens adhaerescens, Bidens africana, Bidens alausensis, Bidens alba, Bidens arenaria, Bidens arenicola, Bidens aurantiaca, Bidens barrancae, Bidens bimucronata, Bidens bonplandii, Bidens brachycarpa, Bidens bullata, Bidens calcicole, Bidens californica, Bidens cannabina, Bidens caracasana, Bidens caucalidea, Bidens cernua, Bidens chilensis, Bidens daucifolica, Bidens deamii, Bidens decussata, Bidens dichotoma, Bidens effuse, Bides exaristata, Bidens fastigiate, Bidens heterodoxa, Bidens*

	hirsuta, Bidens hirta, Bidens hispida, Bidens hybrida, Bidens inermis, Bidens leucantha, Bidens leucanthemus, Bidens minor, Bidens minuscula, Bidens montaubani, Bidens odorata, Bidens orendainae, Bidens orientalis, Bidens paleacea, Bidens pinnata, Bidens pumila, Bidens ramosissima, Bidens reflexa, Bidens rosea, Bidens scandicina, Bidens striata, Bidens sundaica, Bidens taquetii, Bidens trifoliata, Bidens tripartite, Bidens valparadisiaca, Bidens viciosoi, Bidens wallichii, Ceratocephalus pilosis, Coreopsis alba, Coreposis corymbifolia, Coreopsis leucantha, Coreopsis leucorrhiza, Coreopsis multifida, Coreopsis odorata, Glossogyne chinensis, Kerneria dubia, Kerneria pilosa, Kerneria tetragona

Morphology:

This annual herb is invasive plants, therophyte herbs, flower head is discoid or radiate, yellow, white or salmon sterile ray floret, can reach up to 0.3 to 1.0 m in height. Green colour, dorsal decumbent or erect, stems is square shape [76]

Distribution:

This herb is distributed in tropical and subtropical area, easily found in agricultural area on the roadsides.

Traditional uses:

In Africa and China: Juice from Root and whole plant have been used to treat malaria [77]. In northern America latin the leaves infusion is drunk for malaria treatment [55].

Antimalarial Activity:

This plant is reported have antimalarial activity and its activity attributed to flavonoid compound and acetylenes**.** Based on *in vitro* root ethanolic extract against *P. falciparum,* wild type plant has shown the higher activity than cultivated plant with IC_{50} 10.4-17.0 μg/ml**.** Result showed that cultivated plant is less active because they are younger [78]. Flavonoid is found to be the active compounds [79].

Phytochemical list:

Palmitic acid, myristic acid, stearic acid, behenic acid, arachidic acid, oleic acid, elaidic acid, ethyl linoleate, linoleic acid, methyl linoleate, pilosol A, ethyl linoleate, sulfuretin, aurone, butein, okanin, luteolin, apigenin, axillaroside, centaureidin, centaurein, eupatorin, luteoside, quercetin, bicyclogermacrene, germacrene D, E-caryophyllene, Z-γ-bisabolene, β-gurjunene, α-humulene, δ-muurolene, selina-3,7(11)-diene, α-caryophyllene, phytol, phytanic acid, campestrol, phytosterin-B, β-sitosterol, stigmasterol, lupeol, β-amyrin, friedelan-3β-ol, squalene, eugenol, β-carotene, p-coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, pyrocatechin, pyrocatechol, p-vinylguaiacol, vanillin, protocatechuic acid, vanillic acid, gallic acid, aristophyll C, bidenphytin, pheophytin A [77].

Pharmacological Activities:

Anti-inflammatory, immunological disorders, digestive disorder, immunological disorders, digestive disorder, infectious disease, cancer, metabolic syndrome, wound healing [77].

2.9.6. Vernonia cinerea (Cyanthillium cinereum)

Vernicular name:

Common name	Iron weed
Thailand local name	หญ้าดอกขาว Ya dhak khaw
Synonym	*Cacalia cinerea, Conyza cinerea, Cyanopis erigeroides, Eupatorium mysotifolium, Seneciodes cinereum, Serratula*

	cinerea, Vernonia cyanonioides, Vernonia dendigulensis, Vernonia diffusa, Vernonia erigeroides, Vernonia lentii, Vernonia leptophylla, Vernonia montana, Vernonia parviflora, Vernonia physalifolia, Vernonia rhomboides, Vernonia villosa

Morphology:

Herbaceous plant, slender, slightly branched, grooved and ribbed stem. The stems also glaburous, hairy, 10-17 cm in tall and 1-8 mm thick. Greenish brown basal branches with dark green apical. Pinkish or purple flowers, fracture, rounded at the heads and flat-topped corymbs. Leaves may be broadly elliptic or lanceolate, coriaceous or membranous, obtuse or may acutely toothed, dark green, alternate, extipulate, smooth, opposite, the size is 2.5-5 cm in long and 1,8-3,6 cm in wide, have an odor [80, 81].

Distribution:

This herb has a wide range of geographical distribution [82] commonly found in South-East Asia and others tropical regions [83].

Traditional uses

In Cambodia, this herb is widely used to treat fever [83]. Ayuverdic medicine use the whole plant to treat fever [82]. In Chinese medicine, the decoction of whole plant is prepared for treating the fever. Report said that by using alone, it is lack of antiperiodic properties but when combine with quinine in small amounts of doses, it showed helpful to prevent malarial fever [84].

Antimalarial Activity:

Vernolides, a sesquiterpene lactone was reported active against W2 *P. falciparum* with the IC_{50} ranging from 3.5-3.9 µM [83]. Whole plant of this herbs has been tested also in 3D7 and K1 strain of *P. falciparum.* The result show that dichloromethane extract showed IC_{50} 8,42 µg/ml (3D7) and 5.85 µg/ml (K1) [85].

Phytochemicals list:

This herb contains sesquiterpene lactones vernolide-A, vernolide-B, β-sitosterol, lupeol, α-spinasterol, β-amyrin, stigmasterol and phenolic resin in the whole plant. The roots contain α-amyrin, α-amyrin acetate, $^{\delta}$-amyrin acetate, β-amyrin acetate and β-amyrin. The leaveas contain urticifolene, carotenoid lutein, sitosterol [86]. The major constituent of the seed contains fats and saponin: α-spinasterol, arachidic, β-amyrn, β-amyrin acetate, linoleic acid, β-sitosterol, palmitic acid and lupeol. This herbs also contain apigenin, luteolin, quercetin, lupeol acetate [81].

Pharmacological activities:

This herb poses anti-tumor, anti-arthritis, anti-hyperglycemic, antioxidant, anti-microbial [86].

2.9.7. Gynura divaricata

Vernaculare name:

Common name	Chinese gynura
Local name	แป๊ะตำปึง Pae-tum-pong
Synonym	*Cacalia albicans, Cacalia hieracioides, Cacalia incana, Cacalia ovalis, Gynura auriculata, Gynura glabrata, Gynura hemsleyana, Gynura incana, Gynura ovalis, Senecio divaricatus*

Morphology:

Perennial, herbaceous, woody erect procumbent base, fleshy top, can reach up to 50-120 cm. The stems are ribbed [87] sparsely pubescent to glabrescent. The leaves are sessile, dentate margin, may entire or distantly, obtuse or acute, cordate or truncate, auricles. pubescent,1 to 15 cm in long peduncles, 1 to 5 per corymb in capitula, 5-7,5 mm in diameter, 4-5 mm long calycular bracts, phyllaries, sparsely pubescent. Orange to

yellow floret, 9-11 mm in long, exserted part is 2-3 mm in long [88]. Fibrous root, scapose ascending or dried stem are purple tinged [89].

Distribution:

This herb can be found in Africa, Asia and Australia [90].

Traditional Uses:

This herb is being used by traditional Chinese medicine for thousand years because it has pharmacological activities, few or no side effect and low toxicity and well known to being used in fever treatment [91]. Study reveal that this herb contains pyrolizidine alkaloid (integerrimine and urasamine) which must be limited in the usage. WHO regulate that limit doses for these alkaloid 15 lg/kg body weight in per day. Additionally, many of *Gynura* members are consumed as a salads or tempura because they are edible and rich in nutrition [90].

Antimalarial Activity Previous Report:

There is no scientific research report about antimalarial activity.

Phytochemical list:

This herb contains alkaloids, flavonoids phenolic acids, terpenoids, polysaccharides, etc. Pyrolizine alkaloids which have been discovered are integerrimine and urasamine. *G. divaricata* also contain niacin, stigmasterol, daucosterol, flavonoid (quercetin, kaempferol, rutinoside), phenolic acids (chlorogenic acid, coumaroylquinic acid, feruloylquinic acid). The major component of volatile oil is cubenol, sphatulenol, $^{\delta}$-cadinene, cedrene, β-caryophyllene, γ-elemene, phytol, α-caryophyllene, β-farnesene, ledol, n-hexadecanoic acid, copaene [91]. The major volatile oil of this herbs is sesquiterpene β-caryophyllene. The others compound such like o-cymene, limonene, and α-copaene also detected in this plant [92].

Pharmacological Activities:

This herb posses hypoglycaemic activity, anti-hypertension, hypolipidaemic effect, anti-ploriferation, antioxidant, and anti-tumor [91].

2.9.8. Gynura pseudochina

Vernaculare name:

Common name	-
Local name	ว่านมหากาฬ Waan mahaakaan
Synonym	*Cacalia bulbosa, Cacalia maculate, Cacalia purpurascens, Crassocephalum miniatum, Gynura annamenis, Gynura biflora, Gynura bodinieri, Gynura bulbosa, Gynura eximia, Gynura inegrifolia, Gynura miniate, Gynura nudicaulis, Gynura purpurascens, Gynura rusisiensis, Gynura sagittaria, Gynura sinuata, Gynyra somalensis, Gynura variifolia, Senecio biflorus, Senecia crassipes, Senecio miniatus, Senecio pseudochina, Senecio somalensis*

Morphology:

Short herbs can reach up to 10-50 cm in high, erect stem is arising from wide sub-globose tubers. Arrangement of the leaves at the base is rosette, sparsely pubescent or may be glabrescent., truncate or cuneate base, ocute or obtuse apex, exauriculate, the margin is shallowly lobed, coarsely dentate or sinuate, upper most leaves is pinnasetic, upper leaves more dissected than lower leaves. The corolla is red with 20 to 30 number of florets which have orange to yellow colour [89].

Distribution:

Distributed in India, Myanmar, Thailand, China, Bhutan, Nepal, Tropical Africa eastward to Srilanka, and Indonesia [89].

Traditional uses:

The root of this herbs has been used traditionally to treat the fever [93, 94]. In Indonesia this herb is used for treatment dengue fever [95].

Antimalarial Activity:

There is no scientific research report about antimalarial activity.

Phytochemicals list:

The leaves contain **flavonoid**, **saponin**, **tannins**, **triterpenoids**, **steroids**, chlorogenic acid, caffeic acid, para fumaric acid, vanilic acid and p-hydroxy benzoic acid [95]. This herb contains active chemical such as 3,5-di-caffeoylquinic acid, quercetin 3-rutinoside, 5-mono-caffeoylquinic acid and 4,5-di-caffeoylquinic acid [93].

Pharmacological activities:

Anti-coagulant, anti-pyretic, diuretic [95]

2.9.9. Tridax procumbens

Vernacular name:

Common name	Mexican daisy
Local name	ตีนตุ๊กแก Tin tukkae
Synonym	*Amellus pedunculatus, Balbisia canescens, Balbisia divaricate, Balbisia elongata, Balbisia pedunculata, Chrysanthemum procumbens*

Morphology:

Procumbent, woody, can reach up to 60 cm in height. The leaves are ovate lanceolate (2-7 cm), pinnatisect (3 lobes). The flower is small, peduncled head is long, ascending persistent achenes, pubescent, 1,5 to 2,5 long and 9,5 to 1 mm in wide [96].

Distribution:

This herb is naturalized in Asia, Australia and tropical Africa but native in tropical America [97].

Traditional uses:

People in Ghana using this herb to treat malaria [98]. The traditional uses of this herbs for malaria also reported from Guatemala [99]. Leaf juice or paste also being used in India to treat the fever [100].

Antimalarial Activity Previous Report:

Antimalarial assay has been done using SYBR green I method, it showed that methanolic extract from leaves showed inhibition in *P. falciparum* 3D7 with IC_{50} 62 µg/ml [101]. The ethanolic leaves extract from Ghana herbs has been reported give inhibition against *P. falciparum* CQ resistant Dd2 using 3h-hypoxanthine uptake assay with median EC_{50} 121,3 µg/ml [98]. Methanolic extract from whole plant also has been investigated and showed that had inhibition against W2 *P. falciparum* with IC_{50} 15.4 µg/mL [102].

Phytochemicals list:

The previous research reported that glucotureolin, dexamethasone, luteoline, flavone, β-sitosterol, quercetin and glycoside are presented in this herb [103].

Pharmacological activities:

Anti-hyperglicemic, anti-leshmanial, hepatoprotective, antioxidant, anti-fungal, anti-hepertic, anti-inflammatory, anti-bacterial and anticyclooxygenase [104].

2.9.10. Wedelia trilobata / Sphagneticola trilobata

Vernacular name:

Common name	Singapore daisy, creeping oxeye
Local name	กระดุมทองเลื้อย Kradum thong lueai

Synonym	*Sphagneticola trilobata*

Morphology:

This plant are perennial creeping herbs, invasive, form "dense mats", can up to 70 cm in tall. Leaves are green glossy, the underside is paler green, hairy white, serrated margins, may have lateral lobes. Rounded stem and root are arising from the node. Vegetative reproduction with stolon may up to 2 m in length. The flower is solitary, inflorenscences are branched, yellow ray florets, 8 to 13 per head, yellow and tubular central disk. This herb is flowering throughout the year [105].

Distribution:

This plant is native to South America [105] and has distributed in many tropical areas like Burma, China, Ceylon, Florida and West Indies especially at low elevation [106].

Traditional uses:

In Vietnam, aerial part or leaves is being used traditionally to treat fever and malaria [106]. This herbs also is being used in Indonesia to treat malaria[107].

Antimalarial Activity:

Bioassay guide fractionation was used to isolate the sesquiterpenes lactones, Wedelolides A and B from ethanolic extract of the leaves and was reported able to inhibit *P. falciparum* using hypoxanthine-^{3}H assay with IC_{50} 1.9 µg/mL and 4.1 µg/mL, respectively [108].

Phytochemical list:

This herb contains **flavonoid**, **triterpenes**, eudesmane **sesquiterpene lactones** and ent-kaurane **diterpenes**. Flower contain sesquiterpene lactones wedetrilides, ent-kaurane diterpenoid, and cinnamic acid derivatives [106]. Wedelolides sesquiterpene lactones is known to be active compoundfor anti-malarial activity from Vietnamese herbs [108].

Pharmacological activities:

Wound healing, anti-microbial, anti-inflammatory, antioxidant, anticancer and antihelmintic [105].

2.9.11. *Eupatorium odoratum / Chromolaena odorata*

Vernacular name:

Common name	Siam weed, devil weed, christmas bush
Local name	สาบเสือ Sap saea
Synonym	*Chromolaena odorata, Chrysocoma maculate, Chrysocoma volubilis, Eupatorium affine, Eupatorium atriplicifolium, Eupatorium brachiatum, Eupatorium clematitis, Eupatorium conyzoides, Eupatorium dichotomum, Eupatorium divergens, Eupatorium floribundum, Eupatorium graciliflorum, Eupatorium klattii, Eupatorium sabeanum, Eupatorium stigmatosum, Osmia atriplicifolia, Osmia clematitis, Osmia conyzoides, Osmia divergens, Osmia floribunda, Osmia graciliflora, Osmia graciliflorum, Osmia odorata*

Distribution:

This herb can be found in Asia, Australia and West Africa but know to be native to Texas, Mexico, the Caribbean and Florida [109].

Morphology:

Climbing herbaceous perennial weed which can reach up to 6 m. Browny-woody stem at the base while soft and green at the top, fibrous root. The flower is white or pale bluish lilac. Leaves are arrowhead-shaped, opposite (length: 50-120 mm, wide: 30-70 mm). The odor is pungent [109].

Traditional uses:

The root and leaves of this herb are used to treat malaria by people in South western Nigeria [110]. The traditional uses for malaria treatment also reported from southeastern Nigeria [111].

Phytochemical screening:

The leaves, stem bark and root has contained phytochemical such as tannins, alkaloids, flavonoids, terpenoids, saponins and phenolic acids [110].

Antimalarial Activity:

13 dichloromethane fractions from methanolic leaves extract were tested to *P. falciparum* chloroquine sensitive (HB3) and chloroquine resistant (FcM29) by using SYBR Green I method. The result showed that the IC_{50} ranging from 4.8 µg/ml - > 50 µg/ml. The further research showed that the active compound quercetin-5 methyl ether identified show potential for further development against malaria [111].

Pharmacological activity:

Antioxidant, anti-fungal, anti-microbial, insecticide, larvicidal, ovicidal [112].

2.9.12. Artemisia dracunculus

Vernacular name:

Common name	Tarragon, pinon wormwood
Local name	ทาร์รากอน Tarragon
Synonym	*Achiella dracunculus, Artemisia aromatica, Artemisia cernua, Artemisia changaica, Artemisia desertorum, Artemisia dracunculoides, Artemisia glauca, Artemisia inodora, Artemisia nutans, Artemisia nuttalliana, Artemisia redowskyi, Draconia dracunculus, Dracunculus esculentus, Oligosporus dracunculiformis, Oligosporus dracunculus, Oligosporus glaucus*

Distribution:

Originate from Afghanistan, southeastern Russia, western North America, Pakistan, Turkey and Mongolia and introduced to many countries later [113].

Morphology:

Aromatic or may be non-odourous perennial herbs, shruby, rhizomatus, can reach up to 60 to 120 cm in height. Leaves are dark gray to green, shiny, alternate, narrow, linear or lanceolate oblong. Ascending or erect stems, woody, may glabrous or sparsely hairy. Fibrous root, brownish and gnarled. The florets are bisexual and sterile, white to reddish with mostly green sepals, oblong to eliptic while the inner is broad edge ovate. Receptacle is glabrous with yellow corolla [113].

Traditional uses:

There is no report about traditional uses for malaria treatment. But this herb has been used traditionally in India to treat various fevers [114]. This herbs commonly used for flavoring food or aromatherapy [115].

Phytochemical screening:

The main component of oils is ocimene, methyl ethers, myrcene, limonene, α- and β-pinene, linalool and camphene. Flavonoids content are patulin glycosides, quercetin, hydroxycoumarins (scopoletin and herniarin) and isocoumarin (polyenes and artemidin) [113]. Whereas report from [116] showed that major constituent of essential oils from aerial part was terpinolene, methyl chavicol and methyl eugenol. Whereas [61] mentioned that essential oils from this herb contained anethole, menthol, anisol, estragole, anisic acid, limonene, d-sabinene, myrcene, α-phellandrene, ocimene, anisaldehyde, coumarin and β-pinene. Based on the research which has been conducted by [60], leaves of this herb contained artemisinin 0.27 ± 0% with higher content in the leaves.

Antimalarial Activity:

Based on [57], the aerial ethanolic extract using percolation extraction is shown inactive against *P. falciparum* CY27 and K1. The IC_{50} is above 200 µg/ml.

Pharmacological activity:

Carminative, digestive, anti-pyretic, anti-inflammatory, anti-septic, anti-parasitic, anti-mircrobial, anti-pasmodic, anti-helmintic and anti-fungal [113]. Anti-platelet, hepatoprotective, anti-hyperglicemic, hypolipidaemic action, neurotropic activity, antioxidant activity, anti-hypoxic, analgesic, anti-convulsant [114].

2.9.13. Eupatorium capillifolium

Vernacular name:

Common name	Dog fennel
Local name	โกฐจุฬา Kod chulaa
Synonym	*Artemisia capillifolia, Artemisia tenuifolia, Chrysocoma capilacea, Eupatorium foeniculaceum, Eupatorium foeniculoides, Mikania artemisioides, Traganthes tenuifolia*

Distribution:

This herb is native to North America and found primarily in the southeastern United States also can be found in temperate North America, Europe and eastern Asia [117].

Morphology:

Annual or perennial, have a short life, 7- 2 m in tall, stem is clustered and branched, scabrous-hirsute to glabrate basally. The leaves once or twice pinnately divided, leaves division filiform less than 0.5 mm wide, inflorescences racemose, apiculate, enclosing 2-5 flowers, green or less commonly reddish, corolla 2.0-2.5 mm in long white or less commonly reddish, achenes [118]. The leaves are fine-textured, narrow segment and very odorous [117].

Traditional uses:

The infusion of this herbs is widely used by Native Americans to treat the fevers [117].

Antimalarial Activity:

-

Phytochemical list:

Essential oils of this herbs contained camphene, β-phellandrene, α-pinene, $^{\delta}$-4-carene, myrcene, α-phellandrene, β-pinene, limonene, β-ocimene, terpinolene, p-cimene,, bornyl acetate, lavandulol, thymol, methyl ether, germacrene D, α-humulene, γ-eudesmol and β-eudesmol. This herbs also contained alkaloid, flavonoids, triterpenes sesquiterpene lactones and acetylenic compounds [117].

Pharmacological activity:

Anti-microbial, anti-tumor, antioxidant and anti-inflammatory [117]

2.9.14. Artemisia chinensis/ Crossotephium chinense

Vernacular name:

Common name	Chinese wormwood/ Anjenjo
Local name	แอหนัง เบญจมาศเงิน ae nang
Synonym	*Chrysanthemum artemisioides, Crossotephium artemisioides, Tanacetum chinense*

Distribution:

This plant has distributed in Asia [119] but is a native of China [120].

Morphology:

Sub-shrub growing in crevices in the rock in Japan and cultivated in Asia as ornamental plant. Glaucous plant, fleshy leaves [121]. The leaves are tomentose, about a finger

breadth in length, the lower leaves are wedge-shaped and trilobed, while the upper is lanceolate and obtuse. Flower ovate, simple racemes [120].

Traditional uses:

No reported data has been mentioned this plant has been used for malaria or fever treatment. This herbs usually is used for joint pain, windpipe infection, antitoxic, bone arthritis and flu and cough [122].

Antimalarial Activity:

-

Phytochemical list:

The ethanolic extract of the whole plant contained sesquiterpene crossostephin, coumarin biscopoletin, artesin, tanacetin scopoletin and scopolin [123]. Flavonoid aglycone, luteolin, apigenin, hispidulin, chrysoeriol, cilsimaritin, jaceosidin, quercetin 3-methyl ether, chrysosplenol-D, axillarin, cirsiliol, cirsilineol, apometzgerin, nepetin, eupatilin were found in the leaves [119, 124].

Pharmacological activity:

Antioxidant, anti-proliferative, hepatoprotective [125].

2.9.15. Sphaeranthus indicus

Vernacular name:

Common name	
Local name	หญ้าขี้ควาย
Synonym	*Sphaeranthus hirtus, Sphaeranthus mollis*

Distribution:

This plant is distributed in tropical area such as Srilanka, India, Australia, Africa and commonly grow well in the dry waste or agricultural area [126].

Morphology:

This herb is aromatic strongly scented, hairy multibranched which can reach up to 1-2 feet in height, annual erect, branched tapering roots tap roots. Leaves are greenish brown hairy, sessile, sphatulate, obovate, deccurent, sub-acute or rounded, oblong, dentate or spinous serrate margin, and the base is narrow. Leaves size is 2-7 cm in length and 1-1.5 cm in wide. Flowers are soliter pinkish purple, terminal born, globose with clustering heads. Outer flowers are female, while inner flowers are bisexual fertile or sterile. The receptacle is small and naked with the purple, slender corolla. The leaves show a trichome with uni-multicelullar, club and clavate [127, 128].

Traditional uses:

Ayuverdic medicine use this herb to treat fever [128].

Antimalarial Activity:

Previous study by using Thai herbs showed that the hexane and ethyl acetate extract of aerial part exhibited IC_{50} > 90 μg/ml which was mean no activity against K1 *P. falciparum*. However, isolated eudesmanolides sesquiterpene lactones showed active inhibit *P. falciparum* with the IC_{50} 2.32- 6.47 μg/ml [129].

Phytochemical list:

This herbs contain citral, estragole, β-sitosterol, $^{\delta}$-cadinene, methyl chavicol, α-ionione, α-terpinene, β-ionone, geranyl acteate, n-pentacosane, oscimene, eugenol, sphaeranthene, geraniol, sphaeranthol, indicusene, n-triacontanol, para-methoxycinnamaldehyde phenylurethane, sesquiterpene lactone 7α-hydroxyeudesm-4-en-6, 12-olide, sesquiterpene glycoside sphaeranthanolide, flavone 7-O-β-D-diglucoside,

flavon glycoside 7-hydroxy-3′,4′,5,6-tetramethoxy neral, geraniol, geranial, maaliene, linalool, camphor, borneol, indipone, cubenol, α- eudesmol, valianol [127].

Pharmacological activity:

Antifeedant, antihelmintic, analgesic, antipyretic, anti-diabetic, antihyperlipidemic, antioxidant, antimicrobial, antiviral, macrofilaricidal, larvicidal, antioxidant, anxiolytic, central nervous system depressant, anticonsvulsant, mast cell stabilizing activity, anti-arthritic, anti-inflammatory, anti-migratory, anti-proliferative, hypolipidemic activity, nephroprotective, antiprotozoal [127]

2.9.16. Acmella oleracea

Vernacular name

Common name	**Toothache plant**
Local name	
Synonym	*Anacylus pyrethraria, Bidens fervida, Bidens fixa, Bidens oleracea, Cotula pyrethraria, Pyrethrum spilanthus, Spilanthes acmella, Spilanthes fusca, Spilanthes radicans*

Distribution:

This plant is distributed in the tropical and subtropical regions [130].

Morphology:

Stems are erect or decumbent, hairless, reddish. Leaves are simple, opposite, broadly ovate to triangular, the size is 5-11 cm in length and 4-8 cm in wide, margin dentate, base truncate or attenuate, the apex is acute or acuminate. The disk flowers are 400-620 while corolla is yellow and up to 3.5 mm in length. Inflorescence is discoid head, apex acute [131].

Traditional uses:

In Africa and India, people use this plant to treat malaria [132].

Antimalarial Activity Previous Report:

Isolated compounds from this plant including undeca-2E-ene-8,10-diynoic acid isobutylamide and spilanthol were reported have inhibition against *P. falciparum* PFB with IC_{50} 41.4 µg/mL and 16.5 µg/mL while on K1 strain were 16.3 µg/mL and 5.8 µg/mL, respectively [132].

Phytochemicals list:

This herb was reported contain alkaloids, flavonoids, saponins, steroid glycosides and tannins in leaves, stems and flower [133].

Pharmacological activity:

This plant posses a various activity including anaesthetic, analgesic, antipyretic, anti-inflammatory, antifungal, diuretic, vasorelaxant, antioxidant, antimalarial, larvicidal, aphrodisiac, antinociceptive, immunomodulatory, convulsant and bioinsecticidal [130].

CHAPTER III METHODOLOGY

3.1. PHYLOGENETIC MAPPING OF ETHNOMEDICINAL PLANT USED FOR MALARIA AND ITS ASSOCIATED SYMPTOMS

3.1.1. Data collection

A number ethnomedicinal plants list which were used to treat malaria and its associated symptoms such as fever and diarrhea were created through literature search. Plants used for tuberculosis were added to increase the number of data in order to analyze the possible chance of cluster pattern mapping alteration because of the addition of a samples number with the different disease's indication. PubMed, Science direct, Google scholar and Scopus were used as a literature databases source. Published articles in ethnobotanical surveys on relevant disease conducted in various cultures (including Indomalaya and Africa) which presented the usage of plants for treatment were used to create the plant working list database.

Inclusion and exclusion criteria which was adapted and modified from [134] were applied to extract the data: 1). Medicinal plants remedies were excluded, 2). Taxa under same genera was only presented once to avoid visually bias (e.g. *Artemisia* spp represented *A. afra, A. annua, A. gmelini,* and *A. brevifolia*).

3.1.2. Phylogenetic Tree Construction

Phylogenetic tree was constructed using ITS region. The ITS sequences of each medicinal plant were obtained from GenBank in NCBI (National Center for Biotechnology Information). Analysis of the sequences was performed using MEGA X freeware (https://www.megasoftware.net/). Obtained sequences were aligned using MUSCLE multiple sequences alignment while Maximum Likelihood Phylogenetic Test was used to construct the phylogenetic tree by bootstrapping 100 times.

ITOL (Interactive Tree of Life, https://itol.embl.de/) and Adobe Illustrator 2020 were used to create the datasets and annotations of interactive phylogenetic tree. Heatmap datasets was used to obtain the clustered pattern of medicinal plants by applying coding system for each medical used condition (0: no usage, 1: present usage).

3.2. **PLANT COLLECTION AND EXTRACTION**

Plants were collected from various source area in Thailand from November 2019 – January 2020. Identification was done by comparing the morphological characters with the reference. All plant samples were identified by botanist and voucher specimens will be deposited at College of Public Health Sciences, Chulalongkorn University. Selected part of the plant (based on ethnomedicinal data for treating malaria and fever) was collected and then rinsed using flowing tap water followed by oven drying at 50 ^{0}C air dying oven. Dried herbs then grinded into the fine powder.

Extraction was done by exhaustive maceration method using ethanol. The plant powder was soaked in the ethanol and filter using Whatman filter paper No.1. Solution of extract then evaporated to obtain concentrated crude extract. Crude extract was stored in -20 ^{0}C until use.

Table 10. Part used of selected medicinal plants.

Species	Local name	Part used	Note
Artemisia vulgaris	โกฐจุฬาลัมพา Khot chulaa luampuaa	Aerial part	Flower, leaves, stem
Artemisia lactiflora	จิงจูฉ่าย Jing ju chai	Aerial part	Leaves, stem
Artemisia chinensis/ Crossotephium chinense	แอหนัง เบญจมาศเงิน ae nang	Aerial part	Flower, leaves, stem
Artemisia dracunculus	ทาร์รากอน Tarragon	Aerial part	Flower, leaves, stem
Tridax procumbens	ตีนตุ๊กแก Tin tukkae	Aerial part	Flower, leaves, stem
Bidens pilosa	ปืนนกไส้ Puen noksai	Aerial part	Flower, leaves, stem
Wedelia trilobata	กระดุมทองเลื้อย	Aerial part	Flower, leaves, stem

	Kradum thong luaoi		
Ageratum conyzoides	หญ้าสาบแร้ง Ya sap raeng	Aerial part	Flower, leaves, stem
Eupatorium odoratum	สาบเสือ Sap saea	Leaves	Mature leaves
Vernonia cinerea	หญ้าดอกขาว Ya dok khaw	Aerial part	Flower, leaves, stem
Gynura divaricata	แป๊ะตำปึง Pae- tom -pung	Aerial part	Leaves, stem
Gynura pseudochina	ว่านมหากาฬ Waan mahaakaan	Leaves	Mature leaves
Blumea balsamifera	หนาดใหญ่ Nadyai	Leaves	Mature leaves
Eupatorium capillifolium	โกฐจุฬา Kod chulaa	Aerial part	leaves, stem
Sphaeranthus indicus	หญ้าขี้ควาย	Aerial part	Flower, leaves, stem
Acmella oleracea		Aerial part	Flower, leaves, stem

3.3. **PHYTOCHEMICAL SCREENING**

The phytochemical screening was done by qualitative standard method based on [135]:

3.3.1. Phenolics compound test (Ferric chloride test)

1 mL of 5 mg/mL ethanolic extract was added with few drops of 5% ferric chloride solution (**Appendix IV**). The color changing into brown - black indicated the presence of phenolic compounds.

3.3.2. Flavonoid test (Alkaline test)

1 mL of 5 mg/mL ethanolic extract was added with 10% NaOH solution followed with 2M HCL. The intense yellow turned into yellow colorless indicated the presence of flavonoid.

3.3.3. Alkaloid test

a. Alkaloid extraction

10 mg/mL extract solution in chloroform was mixed with 1 ml of 25% NH_3 solution. Vortex mixture, and chloroform layer was obtained from the lower phase of the solution. Chloroform layer then extracted with 2 mL of 1% HCl to get alkaloidal layer (upper phase).

b. Dragendorff's test

1 mL of dragendorff's reagent (Appendix IV) was added into alkaloidal solution. Yellow-orange-red precipitation occurred was indicated to be alkaloids.

c. Wagner's test

1 mL of Wagner's reagent (Appendix IV) was added into the alkaloidal solution. The presence of brown-red precipitate indicated the alkaloids.

3.3.4. Steroid and triterpenes test (Salkowski's test)

1 ml of 2.5 mg/mL extract solution in chloroform was added with few drops of concentrated sulphuric acid (H_2SO_4). The reddish-brown ring which was appeared at the interface of the solution was present if the presence of steroid moiety while the yellow color at the lower phase indicated the presence of triterpenes.

3.3.5. Saponin test (foam test)

Saponin test was performed by using foam test. A tiny amount of crude ethanolic extract was shake vigorously with 5 mL of water. The presence of foam on the top of solution which was stood for at least 30 minutes was indicated the presence of saponins.

3.3.7. Diterpenes test (Copper acetate test)

A 1 mL solution of 5 mg/mL ethanolic extract was added with few drops of 1% copper acetate solution (Appendix IV). The color changing to green emerald indicated the presence of diterpenes.

3.3.8. Lactones (Baljet test)

Lactone moiety was detected using Baljet test refers to [136]. A 5 mg/mL extract solution in ethanol was added with Baljet reagent (Appendix IV) which was prepared freshly. Color shift to red or orange indicated the presence of lactone moiety.

3.4. **ANTIMALARIA ACTIVITY ASSAY**

Antimalarial testing method was performed based on [74] and [58] with modifications. Antimalarial activity assay was done at Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University.

3.4.1. Parasite Culture

In vitro antimalaria activity was evaluated against laboratory-adapted *P. falciparum* 3D7 (chloroquine resistant strain) which was obtained from Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. Preparation of parasites culture was performed by aseptic technique in Biological Safety Cabinet Class II (NUAIRE). Parasites was cultured at 37 ^{0} C, 5% CO_2, 5% O_2, 90% N_2 in media contained human erytrocytes suspended in RPMI 1640 suplemented with 10 % Albumax, 4 mM hypoxanthine and 1 M HEPES with the final pH 7.4-7.2. Parasitemia was mantained between 1-2%. The ring stage synchronized parasites was obtained after treating with 5% D-sorbitol solution. Giemsa staining was used to observe the ring stage of parasites by using optical microscope (AXIO) with 100x1.25 magnification.

3.4.2. Assessment of *in vitro* antimalarial activity assay

Antimalarial activity testing was done using DNA fluorescence based assay. Assay was performed automatically by using Eppendorf epMotion 5075. The 96-well drug plates were dosed with this following serial concentration 100 µg/mL, 25 µg/mL, 6.25 µg/mL, 1.5625 µg/mL and 0.390625 µg/mL of ethanolic extract. After that, an aliquot of parasite inoculum (50 µl) with 2% parasitaemia and 1% haematocrit was added into each well of a 96-well microplate. The 96-well drug plates were incubated at 37 °C under a gas mixture of 5% CO_2, 5% O_2, 90% N_2 for 48 h. 10 nM artemisinin were used as positive control.

After incubation, a 100 µL of fluorescent hemolysis reagent was added to each well and incubated the plates in the dark for 1 hour. Fluorescence intensity was determined at the excitation and emission wavelengths of 485 and 530 nm, respectively. Potency of antimalarial was determined by the calculation of IC_{50}. Antimalarial activity was classified into six class based on [58]:

Potency of antimalarial	IC_{50} (µg/mL)
Very good	<0,1
Good	0.1 -1
Good- moderate	>1-10
Weak	>10-25
Very weak	25-50
Innactive	>100

3.5. **GENETIC DIVERSITY OF ITS REGION**

3.5.1. Plant Genomic DNA Extraction

DNA extraction was done using CTAB method and Plant DNA extraction Kit. For CTAB method, young tissue of leaves was grinded to powder using liquid nitrogen. A sufficient amount of powder was put into sterile 1.5 mL tube by addition of 500 µL of 2xCTAB buffer and incubated in water bath for 1 hour at 65 ^{0}C. Centrifugation was done at 10.000 rpm for 10 min to obtain supernatant (Spectrafuge 16M Labnet Internationals).

Supernatant was transferred to sterile 1.5 mL tube followed by adding 500 µL of chloroform. Vortex the solution, milky green solution was appeared and then centrifuged the solution at 10.000 rpm for 10 min to obtain the aqueous phase in the upper layer. The aqueous phase then transferred into new sterile 1.5 mL tube followed by addition of 500 µL mixture of chloroform: isoamyl acetate (24:1), then vortex the solution. After that, centrifuged with the same protocol and collect the aqueous phase (upper layer) into1.5 mL sterile tube. Into the aqueous phase, 3M sodium acetate pH 5.0 was added in the ratio 1:10 volume then invert the tube. Ice cold absolute ethanol (-20 ^{0}C) was added in 2 times of volume then invert the tube, after that, incubated in -20 ^{0}C for 1 hour. After incubation, centrifuge at the same procedure to obtain the pellet of the DNA. DNA pellet was washed using cold 70% ethanol (4 ^{0}C) in two times repeated. After washing, the ethanol was discarded and dried the DNA pellet in room temperature, dissolved DNA in 100 µL TE buffer and store at -20 ^{0}C for further used.

3.5.2. DNA Concentration and Purity Measurement

Concentration and purity of extracted DNA was measured by using NanoDrop (Thermo Scientific™). Agarose gel electrophoresis was run for checking of genomic DNA, checked the possibly contamination and checked for DNA shearing.

3.5.3. Measurement genomic DNA concentration and Purity using NanoDrop

DNA quantity (ng/µL) and purity (A260/280 & A260/230) was checked using Nano Drop machine (Thermo Scientific™). TE buffer was used as blank.

3.5.4. Agarose gel electrophoresis

1.5 % agarose in 1X TBE buffer solution was prepared for making the gel. After the gel was set, transferred the gel into the electrophoresis machine which already filled with 1X TBE buffer. One µL of loading dye was mixed well by up and down pipetting with 5 µL of DNA sample. Carefully loaded the mixed sample into the well of the gel. 1 kb DNA Ladder (Thermo Scientific™) was used to check the molecular weight of genomic DNA. Set the voltage in 100 V and run the machine.

After running was completed, the gel was stained using ethidium bromide for about 5-10 minutes in dark container and followed by washing the excessive stain with

tap water. GenSys software system (InGenius^3 SynGene) was used for imaging the gel in UV chamber.

3.5.5. Amplification of ITS Region

PCR components were list in this following table:

Components	**Stock solution**	**Final concentration**
PCR Buffer	10 X	1 X
$MgCl_2$	25 mM	2.5 mM
dNTPs	10 Mm	0.2;0.4 mM
ITS5 Forward Primer	10 mM	0.2;0.4 mM
ITS4 Reverse Primer	10 mM	0.2;0.4 mM
Taq polymerase	5 unit/µL	1 unit/µL

Sequence of ITS Primer used:

Primer	Sequence (5'-3')	Tm (^{0}C)
ITS5 Forward	GGAAGTAAAAGTCGTAACAACAAGG	55
ITS4 Reverse	TCCTCCGCTTATTGAGC	56

Premix solution was prepared by mixing all above PCR components without DNA template. *Taq* polymerase (Thermo Fisher Scientific) was added on the last step in cold condition. 19 µL of premix solution was added into PCR tube and followed by addition of 1 µL of DNA sample. Solution was mixed by pipetting up and down. PCR reaction was amplified (Proflex PCR System Applied Biosystems by Life Technologies) using this following protocol:

Amplification Step	**Temperature**	**Time**	**Cycle**
Pre-denaturation	95 ^{0}C	5	1 x
Denaturation	95 ^{0}C	30 s	35 x
Annealing	50/55 ^{0}C	30 s	
Extension	72 ^{0}C	30 s	
Post extension	72 ^{0}C	5 min	1 x

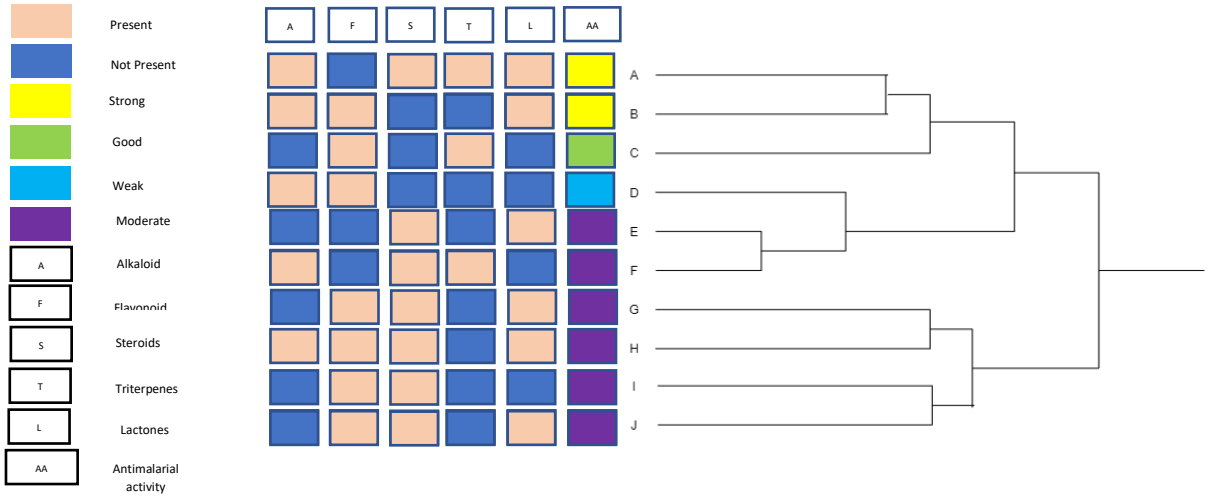
The 1.5% agarose gel electrophoresis was used for checking the PCR products. The product was kept at -4^0 C before used.

3.5.6. ITS region sequencing and Phylogenetic Tree Analyses of selected medicinal plants

The amplified PCR products of selected medicinal plants were sequenced in Apical Scientific Sdn Bhd, Selangor-Malaysia. *Cannabis sativa* was used as an outgroup plant for phylogenetic tree construction. Obtained sequences were aligned with MAFFT (Multiple Alignment using Fast Fourier Transform) by CIPRES portal (www.phylo.org) followed by phylogenetic tree construction using RAxML (Randomized Axelerated Maximum Likelihood) and visualized by using FigTree v.4.0. Sequences alignment was visualized using Jalview 2.10.5. Creating datasets, annotation and make up of interactive phylogenetic tree was performed by ITOL and Adobe Illustrator 2020.

3.6. DATA ANALYSIS

Determination of antimalarial activity of plant extract was categorized as very good, good, good-moderate, weak and very weak based on their IC_{50}. The relation between phylogeny of the species and their activity or phytochemical diversity was performed by descriptive analysis which was shown in this following example diagram:



3.7. **EXPECTED BENEFITS**

3.7.1. This research will provide scientific data about antimalarial activities from Thai herbs which have been used traditionally by people in various region of the world hence will promote to use the herbs which are proven to have activity.

3.7.2. This research will provide insight to use phylogeny as predictive tools to "hit" the group of plant for further bioactivity investigation hence can help to cut the time for selecting the target plants and explore new medicinal plants.

3.8. **RESEARCH BUDGETS**

No	Budgets	Amount	Expenses	Total Expenses
1	**Consumables Laboratory Tools and Supplies**			
	Pippetes, microtubes, tips	N/D	6000	6000
	PCR Kits	N/D	2900	2900
	Whatmann No. 1 Filter Paper	N/D	2000	2000
	Evaporating Bowl	N/D	2000	2000
	Disposable pipettes volumetric (2 ml, 5 ml, 10 ml)	N/D	3000	3000
	Disposable Culture Flask	20 EA	2000	2000
	96 well-plate	100 EA	3800	3800
	Glass slide	1 pack	200	200
	Mask	2 pack	250	250
	Gloves	2 pack	300	300
2	**Phytochemical Screening Reagents**			
	Pottasium Iodide	100 g	5700	5700
	Bishmuth (III) subnitrate	5 g	2500	2500
	Ammonia Solution	100 ml	3500	3500

	Conc. HCl	100 ml	3000	3000
	Conc. H2SO4	100 ml	4200	4200
	Chloroform	1 L	4000	4000
	Ethanol	15 L	1000	1000
	Picric acid	1 GAL	8600	8600
3	**Standard Compounds**			
	Artemisinin	100 mg	3710	3710
	Asiaticoside	1 mg	1500	1500
	Quercetin	10 g	2200	2200
	Quinine	5 g	3100	3100
	Chloroquine	25 g	3300	3300
4	**Antimalarial Assay Parasites culture, Media and Reagents**			
	RPMI 1640	10 L	2200	2200
	HEPES Buffer	25 g	2200	2200
	Albumax	5 g	3300	3300
	Human Erythrocytes	N/D	4500	4500
	Strain 3D7 *P. falciparum*	N/D	1000	1000
	Strain K1 *P. falciparum*	N/D	1000	1000
	D-Sorbitol	100 g	1000	1000
	Giemsa	500 ml	3900	3900
	Fluorescence DNA binding dye	500 µl	5300	5300
	Lysis Buffer	100 ml	2000	2000
5	**Plant Molecular Analysis**			
	Liquid Nitrogen	N/D	500	500
	DNA Extraction Reagent	N/D	6300	6300
	PCR component reagent	1 vial	2300	2300
	Primer	2 vial	5000	5000
	Taq Polymerase enzyme	250 unit	7000	7000
	Agarose Gel	25 g	12500	12500

	Ethidium bromide	10 ml	3200	3200
	Loading dye	2 ml	1200	1200
	DNA ladder	1 vial	6700	6700
	Sequencing facilities	N/D	10000	10000
TOTAL				**149860**

3.9**. TIME SCHEDULE**

Activities	1st year		2nd year	
	Semester 1	Semester 2	Semester 1	Semester 2
Literature Review	←	→		
Proposal Writing And exam		←	→	
Lab work		←	→	
Data analysis			←	→
Thesis Writing			←	→
MS preparation				↔
Thesis examination				↔

CHAPTER IV RESULT

4.1. Combinatorial approach using phylogeny and ethnobotanical data for selecting antimalarial plants target.

A number of 733 medicinal plants list which were used by various culture including Africa (Zimbabwe, West Bengal, Uganda, Nigeria, Senegal, Congo, Ghana, Ivory Coast, Kenya, Limpopo, Madagascar, and Bizana) and Indomalaya (India, Iran, Nepal, Indonesia, Malaysia, Thailand, Bangladesh and Pakistan) were obtained from literature search however only 340 medicinal plants were met the inclusion and exclusion criteria as well as their ITS sequences were available in GenBank NCBI (62 plants for malaria, 65 plants for fever, 56 plants for diarrhea, 44 plants for tuberculosis and 113 plants for multipurpose). Furthermore, this plants list was processed for further analysis.

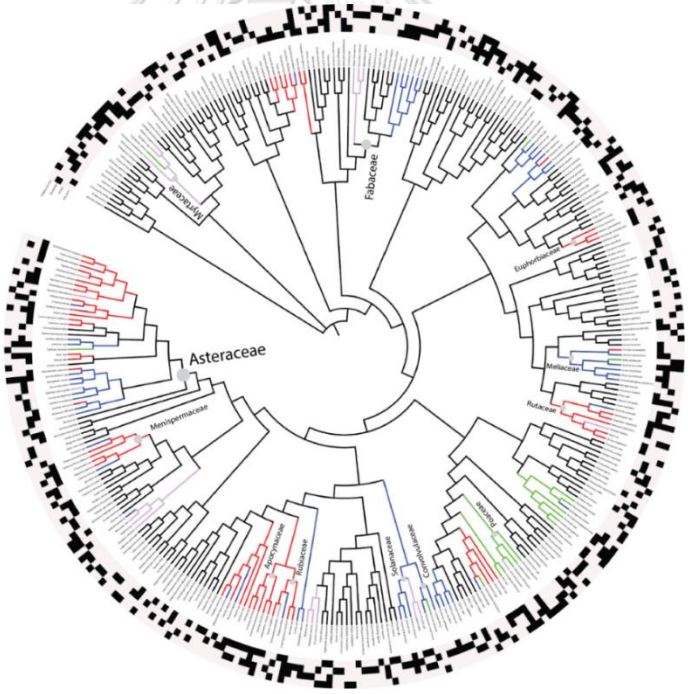


Figure 18. phylogenetic mapping of medicinal plants used for malaria (red), fever (blue), diarrhea (purple) and tuberculosis (green) generated by Maximum Likelihood Test and MUSCLE sequences alignment.

Based on analysis of phylogenetic tree constructed using data of medicinal plants used for treatment of malaria, fever, diarrhea and tuberculosis, the result showed that the medicinal

plants used for malaria mostly clumped in Asteraceae family followed by Rutaceae, Apocynaceae, Rubiaceae and Euphorbiaceae as shown in **Fig 18**. In other hand, plants for fever were shown to be clustered in Asteraceae, Meliaceae, Solanaceae, Convolvulceae and Fabaceae. The clustered pattern for diarrhea treatment showed on the clade of Myrtaceae and Fabaceae. The last, for tuberculosis treatment, the clumping pattern were shown in the Poaceae clade.

After extracting data to only used the malaria and its associated symptoms (fever and diarrhea), clustered pattern of malaria showed to be similar with the pattern showed in the previous analysis before extracting the data as shown in **Fig 19**. The antimalarial plants mostly clumped in Asteraceae along with fever and followed by clumping occurrence in Apocynaceae, Rubiacaeae, Euphorbiaceae, and Rutacaeae. In other hand, clustered pattern medicinal plants for fever were quite different which was not only shown in the clade of Myrtaceae and Fabaceae. In addition, plants for diarrhea treatment were showed different result after data extraction which was shown to be clustered in Rutaceae instead of Myrtaceae and Fabaceae.

Some clustered pattern result after data extraction was shown to be different especially for fever and diarrhea, hence in this study further data extraction for analyzing the clumping pattern using malaria, fever and tuberculosis was performed to observe the consistency result using this approach as shown in **Fig 20**. The plants for malaria treatment was remain the same which was greatly clustered in Asteraceae family, followed by Apocynaceae, Rubiaceae and Euphorbiaceae. In other hand, plants for fever treatment were clumped in Asteraceae and Cucurbitaceae. For tuberculosis treatment, the plants were majorly clumped in Apiaceae clade.

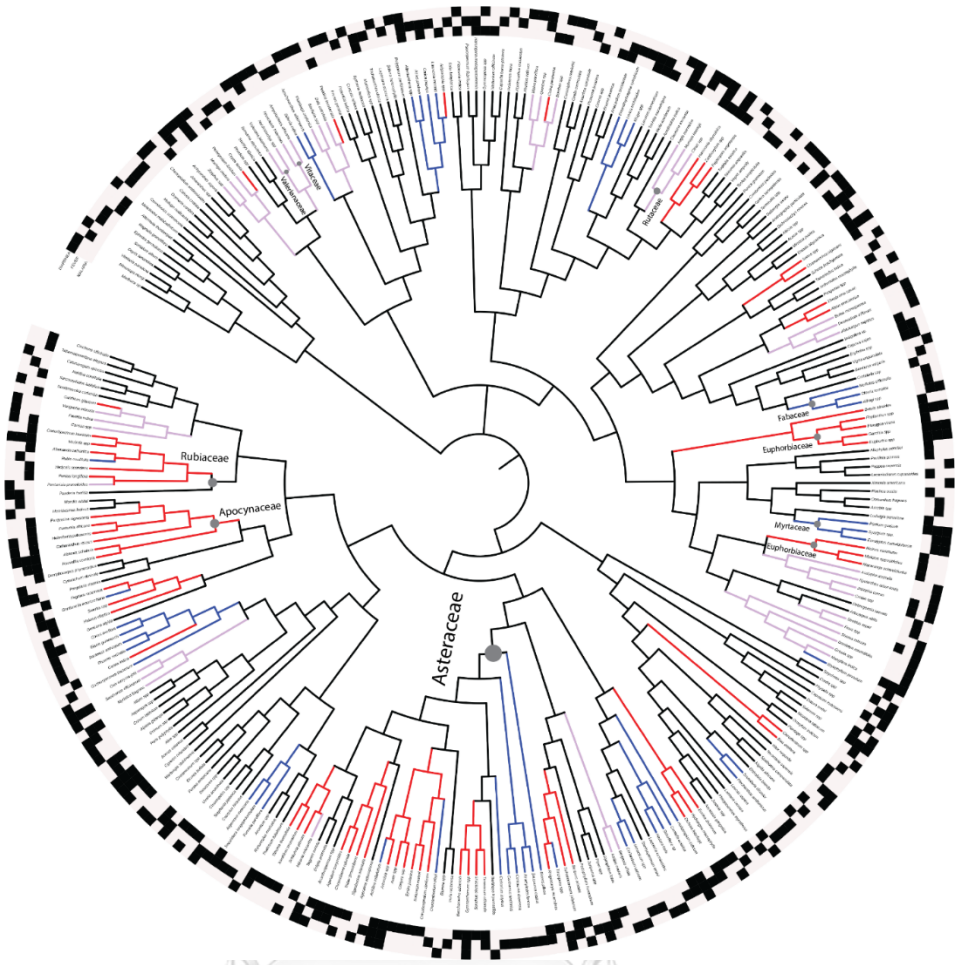


Figure 19. phylogenetic mapping of medicinal plants used for malaria and its associated symptoms (fever and diarrhea) generated by Maximum Likelihood Test and MUSCLE sequences alignment. Plants used for malaria was represented with red color, fever was represented with blue color while diarrhea was represented with purple color.

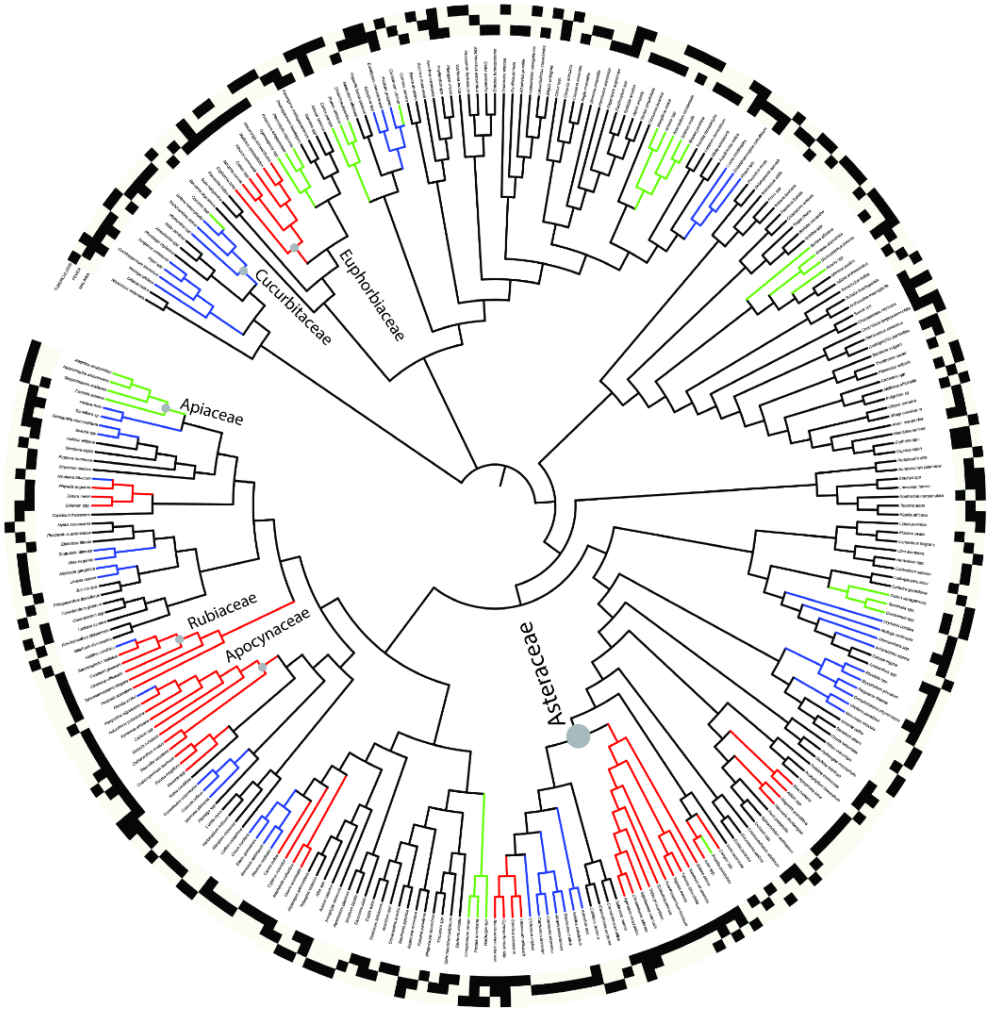


Figure 20 phylogenetic mapping of medicinal plants used for malaria (red), fever (blue) and tuberculosis (green) generated by Maximum Likelihood Test and MUSCLE sequences alignment.

Based on these comparison of phylogenetic tree generated after data extraction, the result revealed that there was a strong clustered pattern signal and consistency for malaria and fever treatment which was shown in Asteraceae family. Accordingly, these data was further analyzed Besides, the other medical category such as diarrhea and tuberculosis were shown to be

inconsistent as shown in **Fig 21**. The plants used for malaria treatment were majorly clustered in Asteroideae.

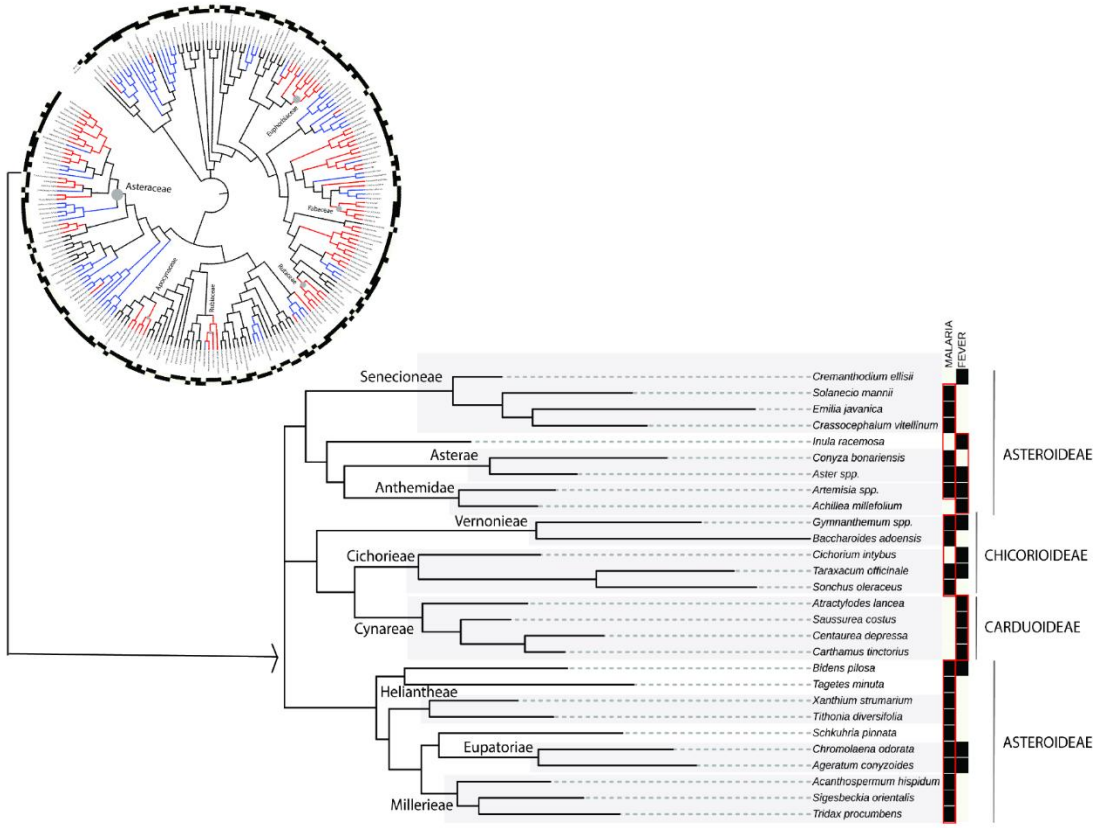


Figure 21. phylogenetic mapping of plants used for malaria (red color) and fever (blue color) generated by Maximum Likelihood Test and MUSCLE sequences alignment.

4.2**. Preliminary phytochemical screening of 16 selected Asteraceae medicinal plants**

Phytochemical screening was performed to determine the presences of phenolic compounds, flavonoids, alkaloids, lactones, diterpenes, triterpenes, steroids and saponins.

Table 11. Preliminary phytochemical screening result of 16 selected Asteraceae medicinal plants

No.	SPECIES	SOURCE OF COLLECTION	PHENOLICS	FLAVONOIDS	ALKALOIDS		TRITERPENES	STEROIDS	LACTONES	DITERPENES	SAPONINS
					Drag.	Wag.					
1.	*A. vulgaris*	Tak Province	++	+	-	+	+	+	+	+	-
2.	*A. lactiflora*	Bangkok	+	+	+	+	+	+	+	+	-
3	*A. dracunculus*	Nakhon Pathom	++	+	-	+	+	-	+	+	-
4.	*A. chinensis*	Northaburi	++	++	-	-	+	+	+	+	-
5.	*A. conyzoides*	Nakhon Pathom	+	+	+	+	+	+	+	+	+
6.	*E. odoratum*	Chiang Mai	++	+	+	+	+	+	+	+	-
7.	*V. cinerea*	Chiang Mai	++	+	+	+	+	+	+	+	-
8.	*W. trilobata*	Bangkok	++	+	-	+	+	+	+	+	+
9.	*T. procumbens*	Chiang Mai	+	+	+	+	+	+	+	+	-
10.	*B. balsamifera*	Chiang Mai	++	+	+	+	+	+	+	+	+
11.	*G. divaricata*	Bangkok	++	+	-	+	-	+	-	-	+
12.	*G. pseudochina*	Bangkok	++	+	+	+	-	-	-	+	-
13.	*B. pilosa*	Chiang Mai	++	++	+	+	+	+	+	+	+
14.	*E. capilifolium*	Northaburi	+	+	+	+	+	+	+	+	-
15.	*S. indicus*	Mukdahan	++	++	+	+	+	-	+	+	-
16.	*A. oleracea*	Nakhon Sithumarat	++	+	+	+	+	+	-	+	-

Note: +: presence (additional + sign was showed more intense color); -: not present; Drag.: Dragendorff's test; Wag.: Wagner's test

The result showed that in certain concentration, all plant tested contained phenolics and flavonoids compound, while the others compound was shown to be vary depend on the species.

Alkaloids screening test was performed using Dragendorff's and Wagner's test. Based on the Dragendorff's test result, most of species tested was containing alkaloid including *A. lactiflora, A. conyzoides, E.odoratum, V. cinerea, T. procumbens, B. balsamifera, G. pseudochina, B. pilosa, E. capilifolium, S. indicus*, and *A. oleracea*. All positive result generated from Dragendorff's test showed similar result when tested with the Wagner's test. However, some species which were shown negative with Dragendorff's test were shown to be positive after testing with Wagner's test including *A. vulgalis, A. dracunculus, W. trilobata* and *G. divaricate*

Salkowski's test was used to determine the presence of triterpenes and steroid moiety. Result indicated that almost all tested plants showed the presence of triterpenes excluding *G. divaricata* and *G. pseudochina.* In other hand, for *A. dracunculus, G. divaricata* and *S. indicus* showed no presences of steroid moiety while the other species indicating the presence of them.

Saponin was determined using foam test by observing the production of persistent foam after shaking vigorously. Based on the result, only several species which was showed the presence of saponin while most species weren't showed indication the presence of this compound. *A. conyzoides, W. trilobata*, *B. balsamifera, G, divaricata*, and *B. pilosa* showed the presences of saponin while the others were not.

Diterpenes was determined using copper acetate test and positive result showed color changing into green emerald. Based on the test result, all tested species showed positive result for this test except *G. divaricata.*

Lactones was detected using Baljet's test which is containing picric acid in alkaline solution. The changing of the solution into orange to red color was indicated the presences of the lactonic compound. Cardiac glycosides and sesquiterpene lactones might give the positive result by containing the lactone moiety in their structures. Hence, this result merely gave the guide for further phytochemical investigation. In certain concentration tested, almost all species was showed positive result for lactones test except *A. oleracea*.

4.3. **Internal Transcribed Spacer (ITS) region of 16 selected Asteraceae medicinal plants**

ITS region was successfully amplified using ITS4 and ITS5 forward and reverse universal primers as shown in **Fig 23**. Amplified ITS region of tested plants then were sequenced followed by phylogenetic analyses.

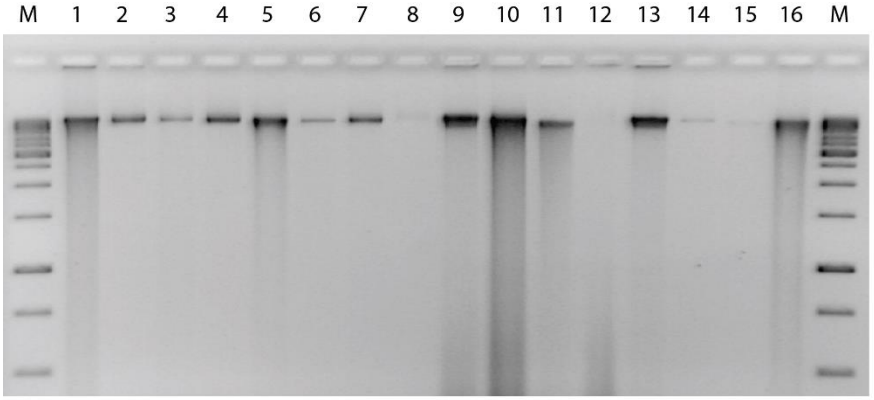


Figure 22. Genomic DNA of 16 plants on 1.5% agarose gel (M=1 kb marker)

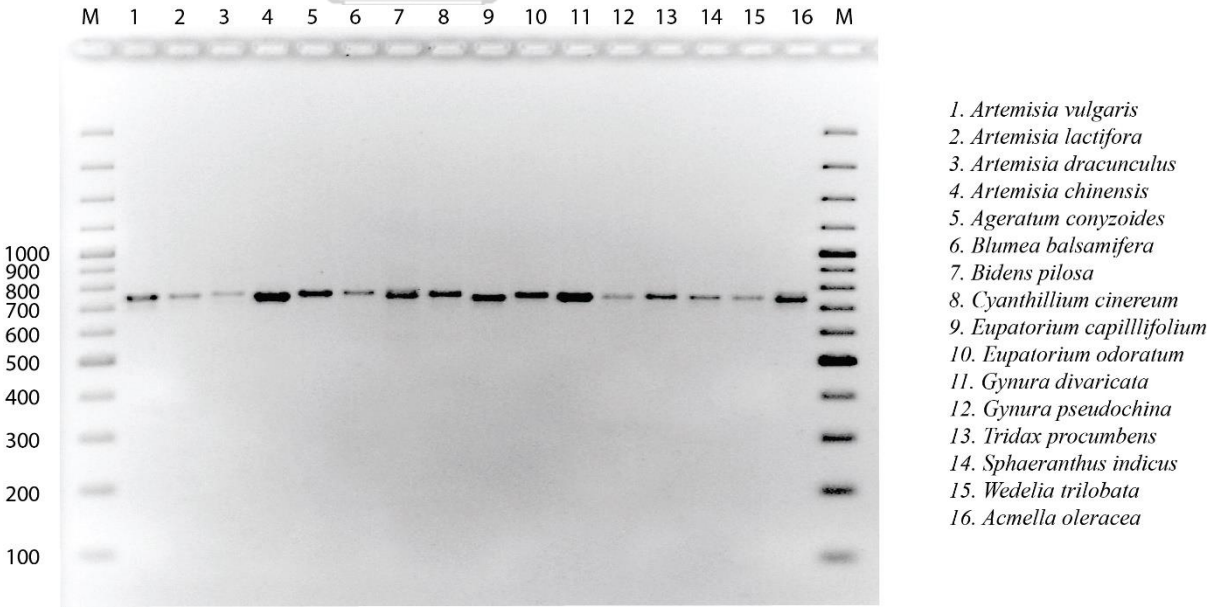
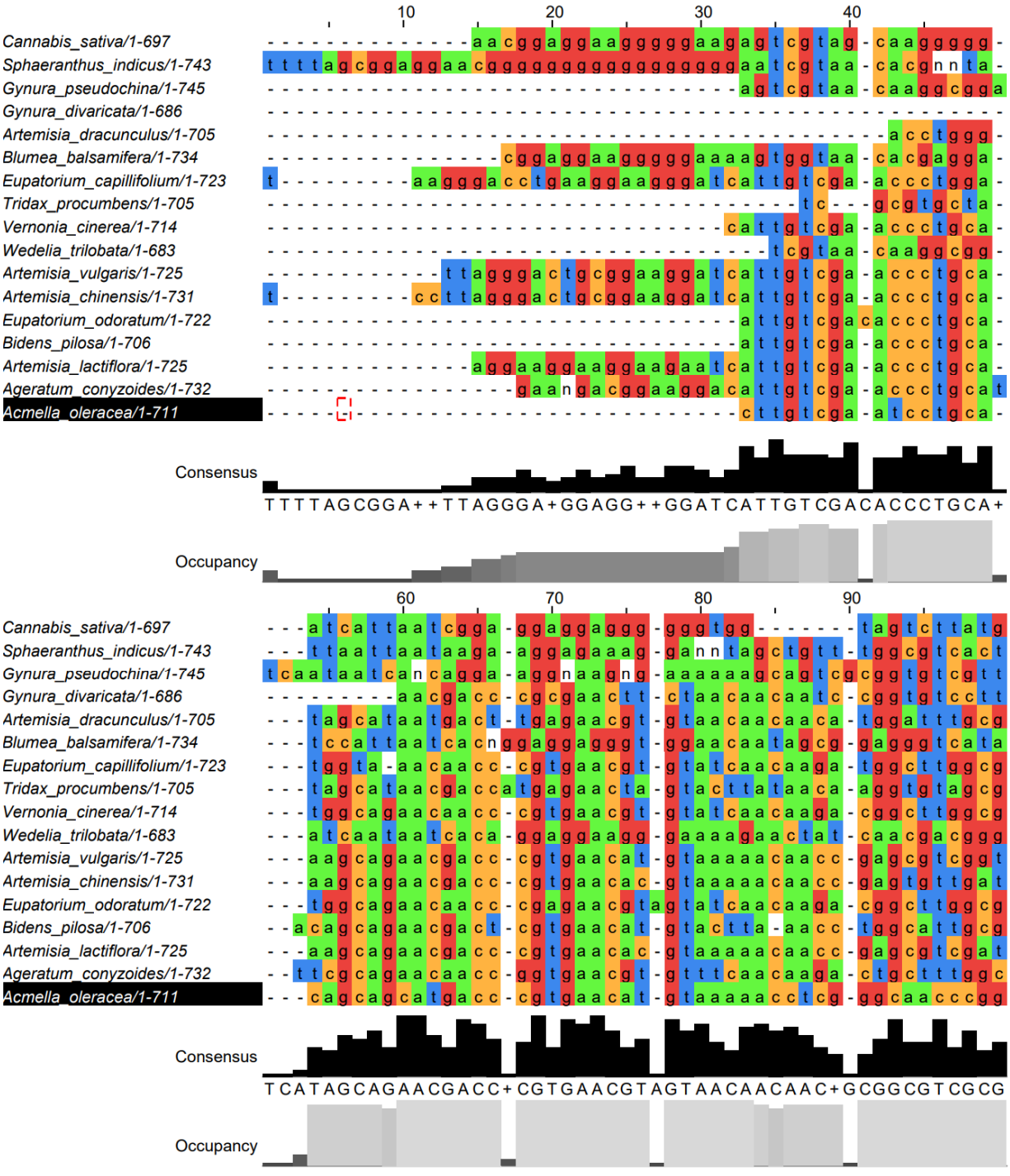
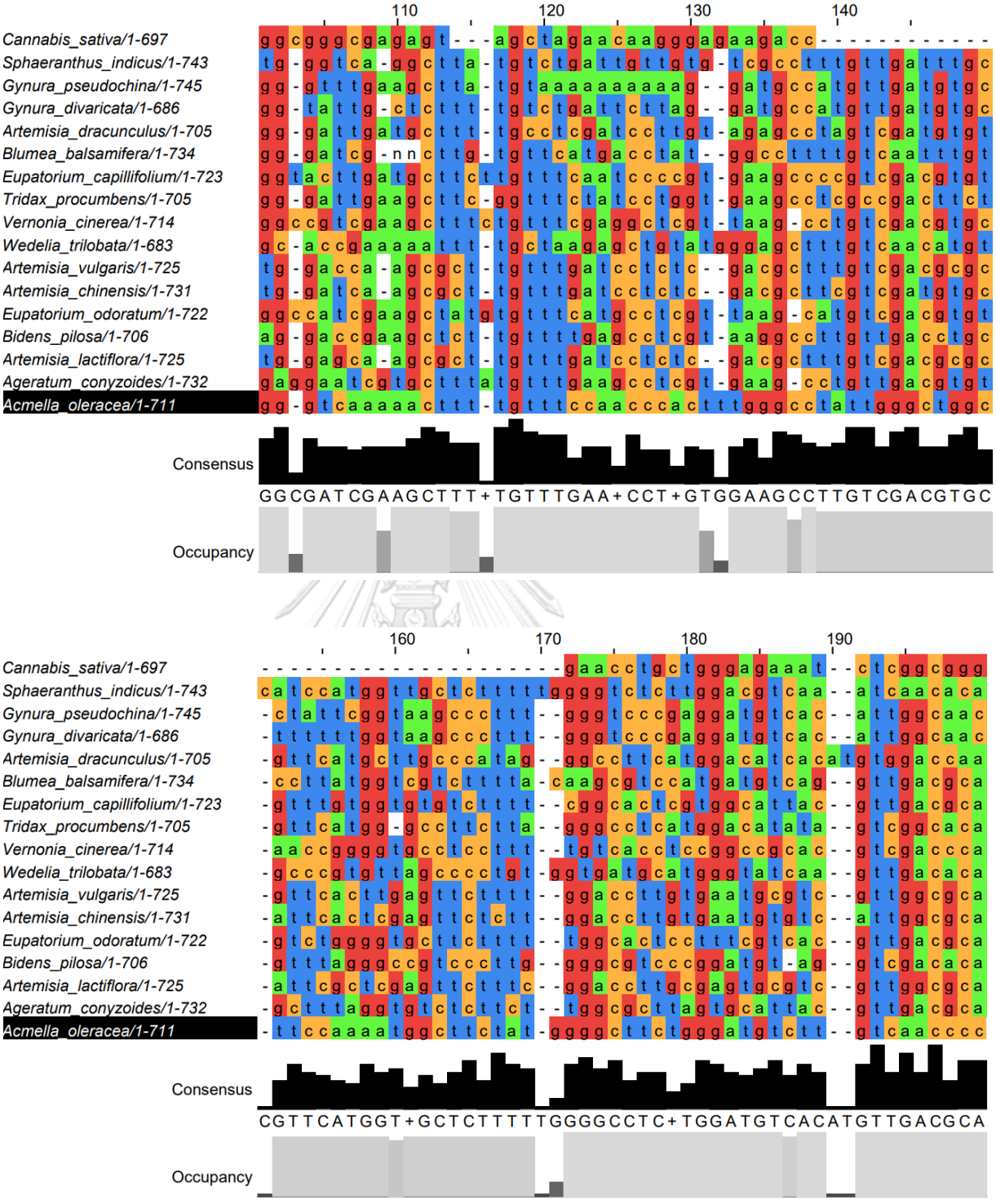
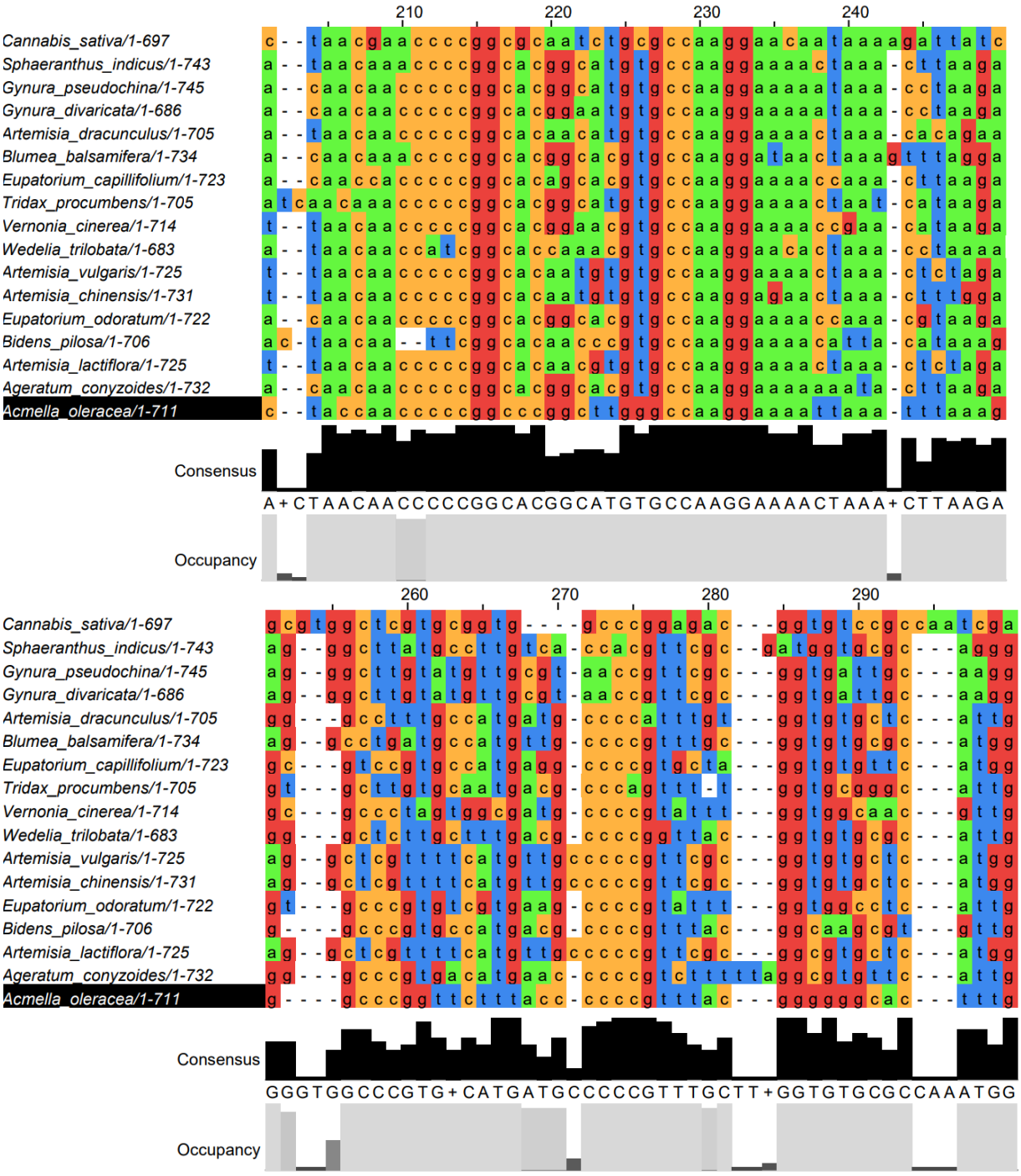


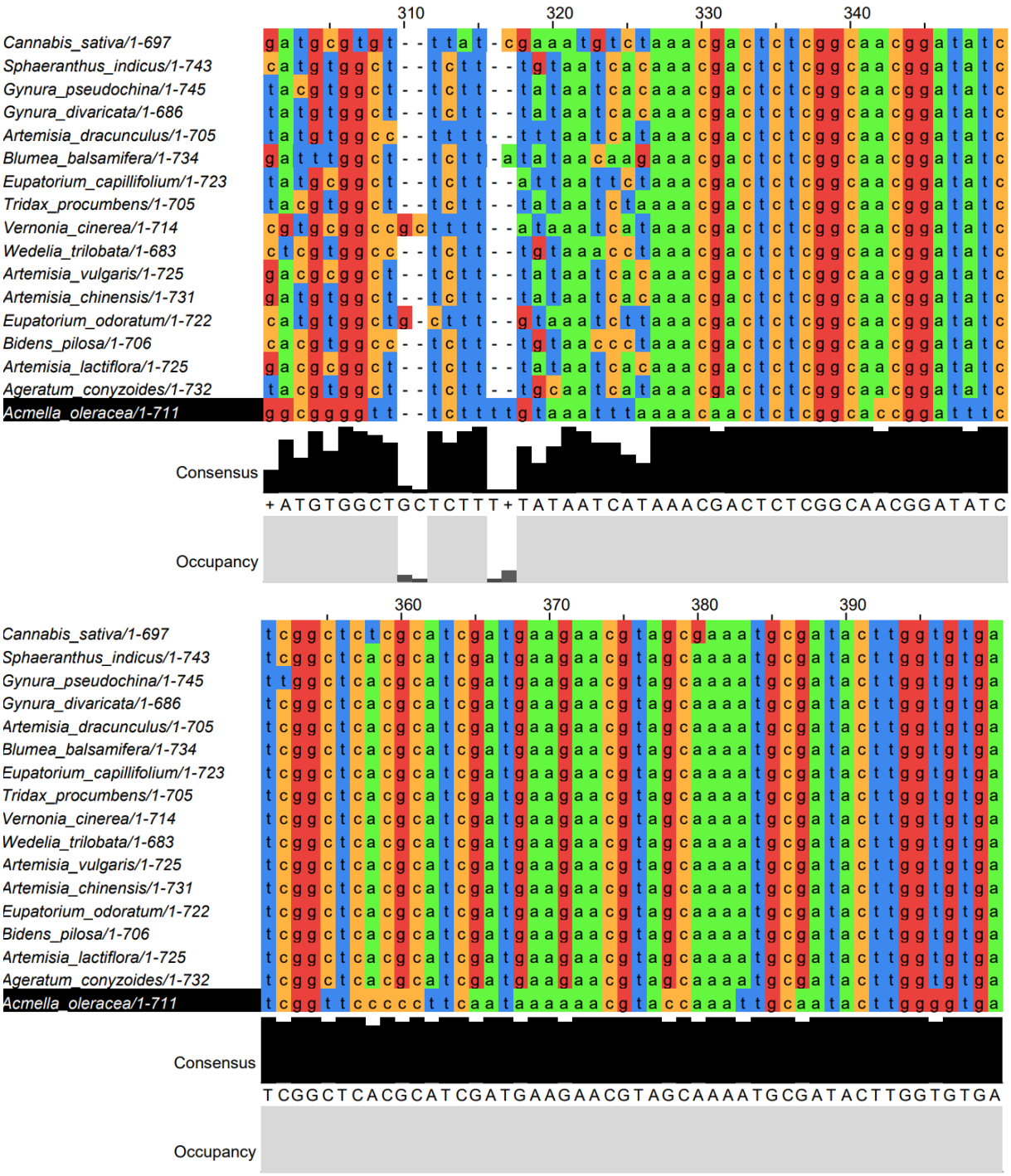
Figure 23. ITS region PCR products of 16 tested plants on 1.5% agarose gel (M=100 bp marker)

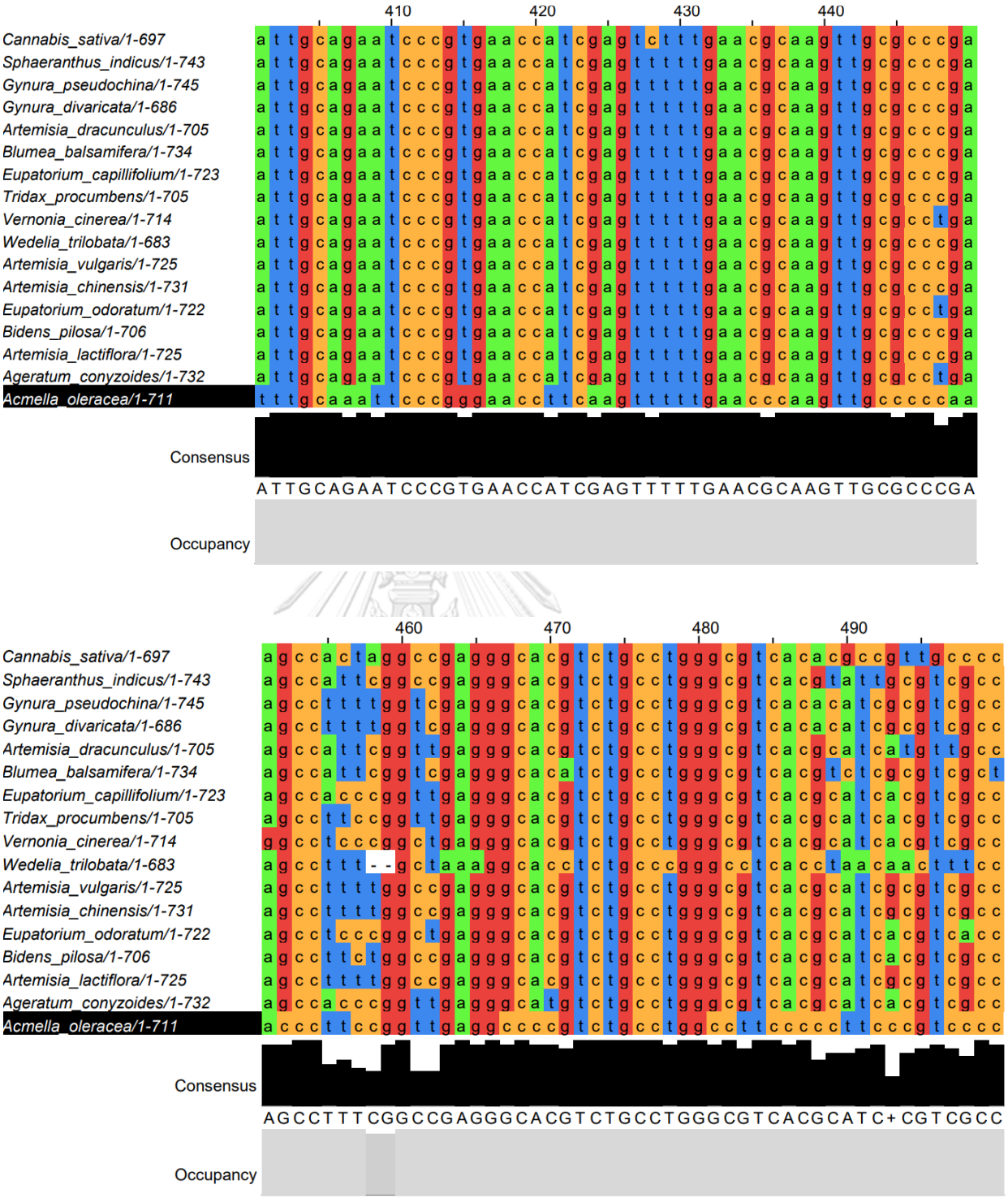
All obtained ITS sequences data with the size of 683-745 bp were aligned using MAFFT then visualized using Jalview v2.10.5 to observe the base similarity and differences. The sequences alignment with the outgroup species (*C. sativa*) were shown in figure below:

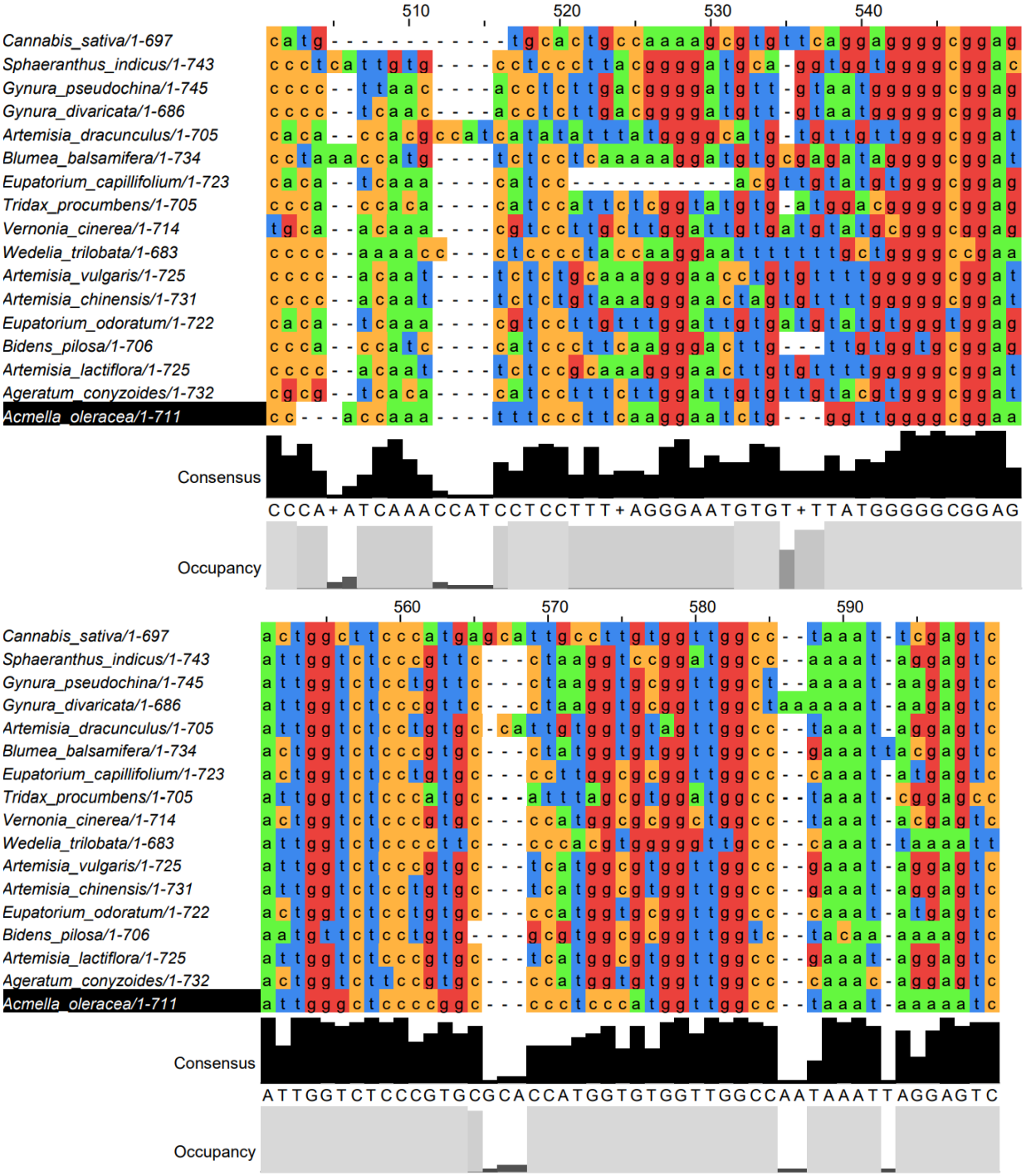


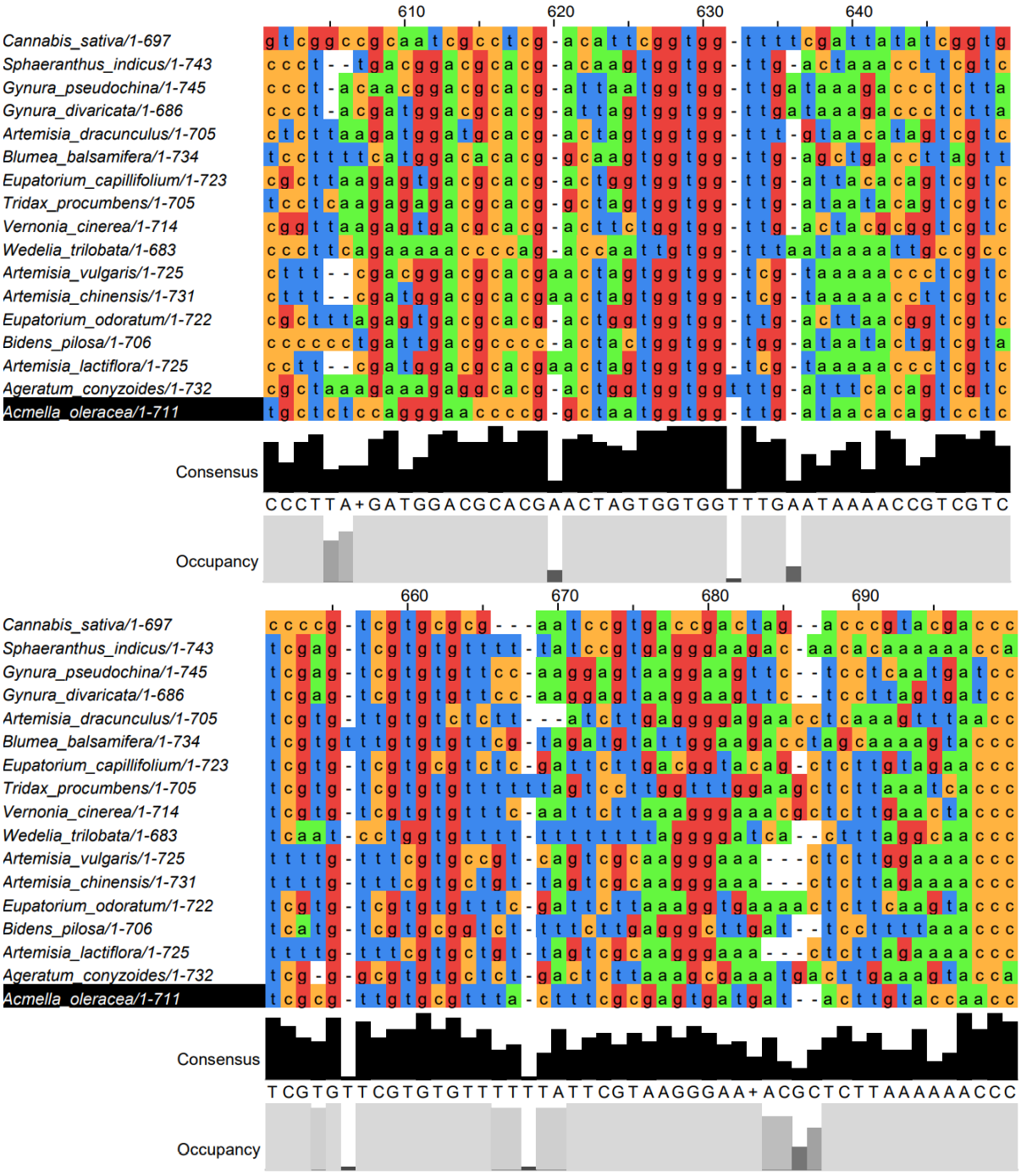

110
120
130
140
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
GGCGATCGAAGCTTT+TGTTTGAA+CCT+GTGGAAGCCTTGTCGACGTGC
Occupancy
160
170
180
190
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
CGTTCATGGT+GCTCTTTTTGGGGCCTC+TGGATGTCACATGTTGACGCA
Occupancy

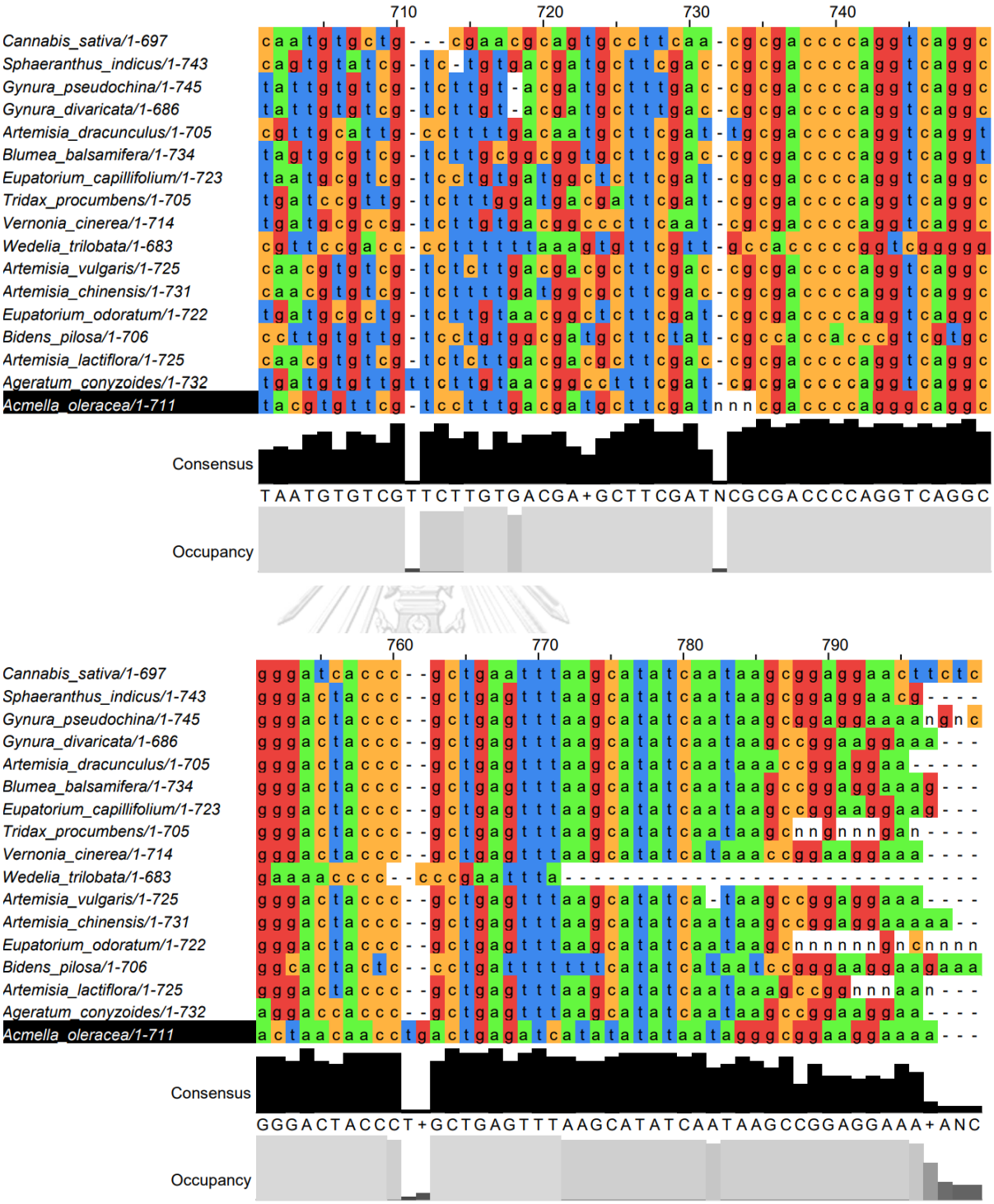

210
220
230
240
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
A+CTAACAACCCCCGGCACGGCATGTGCCAAGGAAAACTAAA+CTTAAGA
Occupancy
260
270
280
290
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
GGGTGGCCCGTG+CATGATGCCCCCGTTTGCTT+GGTGTGCGCCAAATGG
Occupancy


310
320
330
340
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
+ATGTGGCTGCTCTTT+TATAATCATAAACGACTCTCGGCAACGGATATC
Occupancy
360
370
380
390
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
TCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTGTGA
Occupancy


410
420
430
440
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
ATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGA
Occupancy
460
470
480
490
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
AGCCTTTCGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATC+CGTCGCC
Occupancy


510
520
530
540
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
CCCA+ATCAAACCATCCTCCTTT+AGGGAATGTGT+TTATGGGGGCGGAG
Occupancy
560
570
580
590
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
ATTGGTCTCCCGTGCGCACCATGGTGTGGTTGGCCAATAAATTAGGAGTC
Occupancy


610
620
630
640
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
CCCTTA+GATGGACGCACGAACTAGTGGTGGTTTGAATAAAACCGTCGTC
Occupancy
660
670
680
690
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
TCGTGTTCGTGTGTTTTTTATTCGTAAGGGAA+ACGCTCTTAAAAAACCC
Occupancy


710
720
730
740
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
TAATGTGTCGTTCTTGTGACGA+GCTTCGATNCGCGACCCCAGGTCAGGC
Occupancy
760
770
780
790
Cannabis_sativa/1-697
Sphaeranthus_indicus/1-743
Gynura_pseudochina/1-745
Gynura_divaricata/1-686
Artemisia_dracunculus/1-705
Blumea_balsamifera/1-734
Eupatorium_capillifolium/1-723
Tridax_procumbens/1-705
Vernonia_cinerea/1-714
Wedelia_trilobata/1-683
Artemisia_vulgaris/1-725
Artemisia_chinensis/1-731
Eupatorium_odoratum/1-722
Bidens_pilosa/1-706
Artemisia_lactiflora/1-725
Ageratum_conyzoides/1-732
Acmella_oleracea/1-711
Consensus
GGGACTACCCT+GCTGAGTTTAAGCATATCAATAAGCCGGAGGAAA+ANC
Occupancy

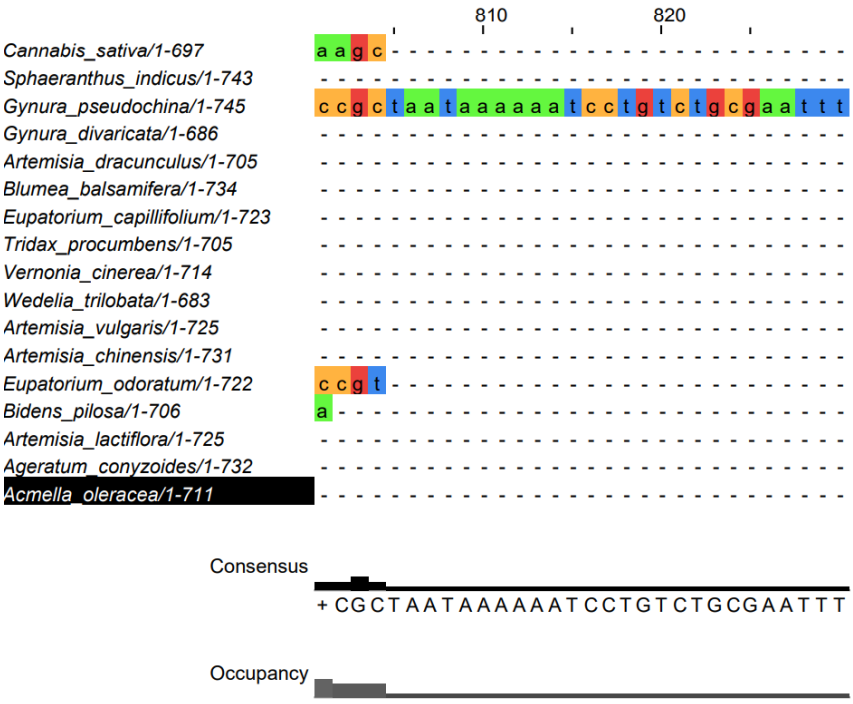


Figure 24. Sequence alignment of ITS region of selected Asteraceae medicinal plants

4.4. **Antimalarial activity of 16 selected Asteraceae medicinal plants**

16 ethanolic extract of selected species from Asteraceae family were tested against 3D7 *P. falciparum* using DNA fluorescence-based assay and showed the activity by the table below:

Table 12. IC_{50} (µg/mL) ethanolic extract of 16 selected Asteraceae plants.

No	Species	Traditional uses	Indigenous culture	Tested part used	IC_{50} against 3D7 *P. falciparum* (µg/mL)	Category
1.	*Artemisia vulgaris*	Malaria and fever	Northern America Latin	Aerial part (flower, leaves, stem)	13.37	Weak
2.	*Artemisia lactiflora*	Heat clearing	Chaosan China	Aerial part (leaves, stem)	6938	Inactive
3.	*Artemisia dracunculus*	Fever	India	Aerial part (flower, leaves, stem)	437.30	Inactive
4.	*Artemisia chinensis*	None	None	Aerial part (flower, leaves, stem)	18.30	Weak
5.	*Ageratum conyzoides*	Fever	Asia, South America and	Aerial part (flower, leaves, stem)	377023	Inactive

			Africa			
6.	*Blumea balsamifera*	Malaria and fever	Malaysia, Vietnam	Leaves	19.19	Weak
7.	*Bidens pilosa*	Malaria	Africa, China, Northern America Latin	Aerial part (flower, leaves, stem)	7033	Inactive
8.	*Vernonia cinerea*	Malaria and fever	Cambodia, Ayuverda, China	Aerial part (flower, leaves, stem)	29.17	Very weak
9.	*Eupatorium capillifolium*	Fever	Native american	Aerial part (leaves, stem)	31.30	Very weak
10.	*Eupatorium odoratum*	Malaria	South western and eastern Nigeria	Leaves	150.40	Inactive
11.	*Gynura divaricata*	Fever	China	Aerial part (leaves, stem)	8194	Inactive
12.	*Gynura pseudochina*	Fever	Indonesia	Leaves	1965	Inactive
13.	*Tridax procumbens*	Malaria and fever	Ghana, Guatemala, India	Aerial part (flower, leaves, stem)	14.93	Weak
14.	*Sphaeranthus indicus*	Fever	Ayuverda	Aerial part (flower, leaves, stem)	6.586	Good-moderate
15.	*Wedelia trilobata*	Malaria and fever	Vietnam, Indonesia	Aerial part (flower, leaves, stem)	29.12	Very weak
16.	*Acmella oleracea*	Malaria	India, Africa	Aerial part (flower, leaves, stem)	N/D	Unstable
	IC_{50} ARTEMISININ: 19.91 nM					

According to the result, among all 16 tested plants, only one plant showed good-moderate activity which was *S. indicus* with the IC_{50} 6.586 µg/mL. This plant is notably as herbal treatment for fever in some cultures. In other hand, our result revealed that all species tested which are used as the herbal medicine for treating malaria showed to exhibit weak-very weak activity against 3D7 *P. falciparum* even inactive which were shown by *B. pilosa* and *E. odoratum*. By using this approach, the new medicinal property has discovered from *A. chinensis* which is not used as malaria or fever treatment traditionally and has exhibited weak activity with

the IC_{50} 18.30. This species was chosen because it is closed related (under the same genera) with the artemisinin producing species, *A. annua*.

4.5. **Clustered pattern on phylogeny of 16 selected Asteraceae medicinal plants with traditional uses, phytochemical and antimalarial activity against 3D7 *P. falciparum***

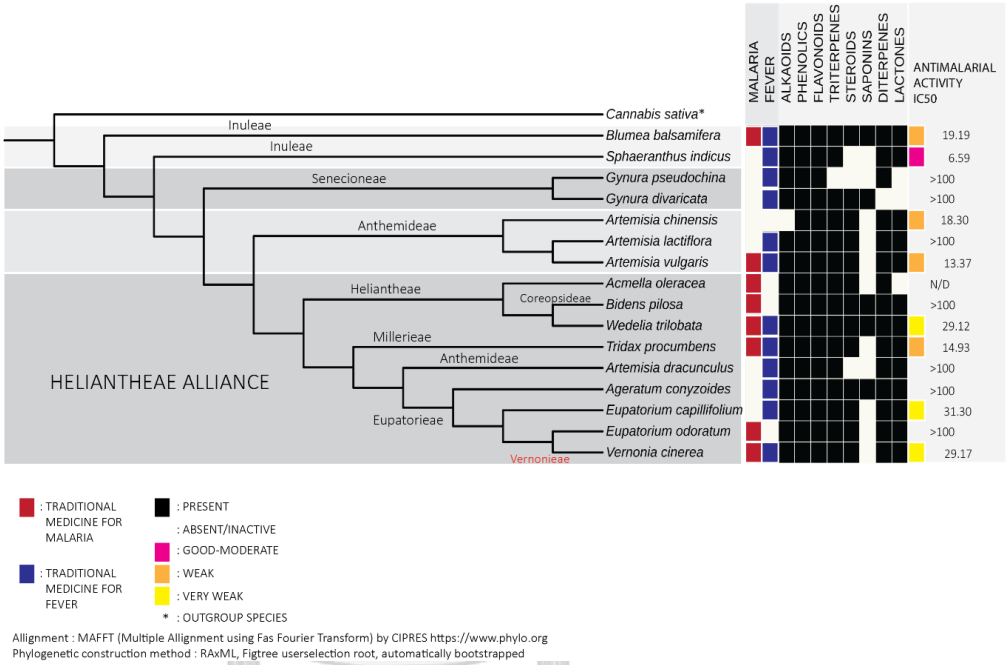


Figure 25. phylogeny, phytochemicals and antimalarial activities of 16 ethanolic extracts of medicinal plants generated from combinatorial phylogeny based on ITS sequences data and ethnobotanical bioprospecting.

Constructed phylogenetic tree by using ITS region showed to be able to separate the species into their clade tribe based on current classification described in Appendix I. However, some contradictive result has occurred in species of *Vernonia cinerea, Bidens pilosa and Artemisia dracunculus*. In the classification of Asteraceae, *V. cinerea* is grouped in tribe Vernonieae-subfamily Chicorioideae while by using ITS region this species was grouped in the tribe of Eupatorieae which is belong to subfamily Asteroideae. *Artemisia dracunculus* which is supposed to be in the clade of Anthemideae along with other *Artemisia* species showed to be in grouped in other clade in Heliantheae

alliance. In other hand, *Bidens pilosa* which is belong to Coreopsideae tribe was in clade in the group of Heliantheae tribe.

In this study, we tried to investigate the clustered signal of antimalarial activity and phytochemicals in the phylogeny of tested plants as shown in **Fig 25**. Bioactivity scattered pattern was shown to be occurred. However, according to the result, plants belong to Heliantheae alliance tribe showed to have very weak-inactive antimalarial activity.

CHAPTER V DISCUSSION

The phylogeny may become promising tools to hit the hot nodes which showed the significantly over-represented clustered clade compared to the rest of the clade in the phylogenetic tree. Previous conducted study aimed to discover the promising candidate plants for screening the antibacterial properties using phylogeny bioprospecting has revealed that similar mechanism of action was shown on all *Berberis* species caused by the alkaloid berberine bearing in the species. Several plant families including Fabaceae, Lauraceae, Combretaceae, Lamiaceae, Cupressaceae, Zingiberaceae and Myrtaceae were highlighted in the phylogenetic tree. Prediction of the promising phytochemical then was performed by investigating the typical chemicals in each family highlighted in the tree and showed that some highlighted family (e.g. Cupressaceae, Myrtaceae and Combretaceae) mostly associated with the inhibition of quorum sensing or biofilm inhibition [137].

Based on the mentioned various research which have supported that close related species may shared similar bioactivity and phytochemical, hence we investigated the clustering pattern in malarial and its associated symptoms disease including fever and diarrhea by retrieving ITS sequence data from the gene bank. Plants for tuberculosis were added into the data to observe the consistency of the clustered pattern by adding the bigger data by using different symptoms of malaria.

In this study, ITS region has been used to construct the phylogenetic tree. ITS region is known to be valuable in phylogenetic analyses in interspecies and intergeneric level of eukaryotes including angiosperms. ITS which is highly repeated region in plant nuclear genomes lead the amplification and sequencing are easy to perform due to its high copy number and small size (<700 bp). This region also undergoes rapid concerted evolution which lead an accuracy of phylogeny reconstruction among species. However, sometimes the non-homologous copies are present with the mutation which may generate small variation within a species [138].

Multiple sequences alignment (MSA) is the method to align more than 2 biological sequences which can be categorized into two including evolution-based method and similarity-based method [139]. In this study, MUSCLE and MAFFT were used as a method to align the sequences. MUSCLE and MAFFT are the progressive alignment program which use guide tree re-estimation. MUSCLE use log expectation score to align the sequences [140]. In other hand, MAFFT as the similarity-based method using the fast fourier transform which allow the rapid detection of the homologous sequences. This method assumes that the sequences are descended from common ancestor and all homologous [139, 141].

According to the generated phylogenetic tree from secondary data obtained from the NCBI gene bank by using MUSCLE sequence alignment and Maximum Likelihood Phylogenetic Test, consistency of strong signal clumping patten in both malaria and fever medical condition was shown in Asteraceae which is known as the family of antimalarial artemisinin producing species (*A. annua*) **(Fig 18-21)**. Hence, this plant family is needed to be further investigate in the laboratory testing.

16 selected medicinal plants indigenous to Thailand were selected based on the traditional used for malaria and fever treatment. In other hand, *A. chinensis* which not been used for malaria and associated symptoms treatment was chosen due to the close relatedness with *A. annua* (under the same genera). Selection of the part of the plants was considered based on the traditional uses. Phylogenetic tree construction of 16 selected medicinal plants was performed by using MAFFT sequence alignment and RAxML (rapid accelerated maximum likelihood) test with automatic bootstrapping.

In this study, *Cannabis sativa* which is the member of family Cannabaceae, Order Rosales was chosen as an outgroup plant due to the genetic distance with the query sequences. The outgroup plant was used to help placing the root in the phylogenetic tree. Based on this result, phylogenetic tree constructed by ITS region showed some contradiction compared to the current Asteraceae classification system as mentioned in **Fig 25** and **Appendix I**. This may be happened because of the possible occurrence of mutation such like deletion and insertion hence may lead the variation within a species. Besides, a small number of our tested samples also may become the other factors

included. According to [142], phylogenetic analyses of family level using ITS sequences data encounter limitation due to the size of the region and the number of information phylogeny hence this sequence may not be ideal for family level. Large sample become a key factor for successfulness of family level phylogenetic analysis using ITS data. In other hand, *Vernonia cinerea* which is supposed to be separated from the clade of Asteroideae subfamily has a taxa synonym *Eupatorium mysotifolium* hence might be possible to be found in the Eupatoriea tribe clade.

In addition, the method used to construct the phylogenetic tree which is maximum likelihood method has a purpose to represent the best tree among the analyzed data not the true tree which is mean that the constructed phylogenetic tree is not always represent the true phylogeny in nature. Commonly, Bayesian inference method is used to represent the true tree. However, the maximum likelihood method is known to overcome if the sequences data are not good or have high variability.

Asteraceae is known to be the largest family of flowering plants which is basically synthesizes the flavonoids, polyacetylenes and terpenoids. In addition, sesquiterpene lactones is the typical compound in this family hence has been used as taxonomic marker [143-145]. Sesquiterpene lactones (SLs) is the C-15 terpenes which is bearing α-methylene-γ-lactone and known to have ability to trap nucleophilic active site of the target enzyme due to their electrophilic moiety property (exocyclic enoate) [73][146]. Antimalarial drug artemisinin is SLs with endoperoxide bridge and the structure is known to be a key for mechanism of action for combating the parasites by producing the radical species inside of the parasite's cell [147, 148]. The SLs are synthesised from common precursor and are known to be predominate in various tribe of Asteraceae including Anthemideae, Senecioneae and Inuleae [149]. Similar chemical structure of SLs may be shared between closed related species (e.g. members of Vernonieae synthesize similar type of guaianolides, members of Eupatorieae produced same type of guaianolides and germacranolides, Inuleae and Heliantheae tribe shared similar skeleton types, etc.) [150].

According to preliminary phytochemicals screening result as shown in **Table 11**, it showed that all tested plant containing phenolic compound groups including

flavonoids. This may be happened because flavonoids are the ubiquitous secondary metabolites which are commonly produced as an adaptive response to the common environmental factors such as UV light exposure. Based on [151] broad range of phenolic compounds (including flavonoids, hydroxycinnamic acid, lignin or tannin) act as UV-B protectant and flavonoid is known to be the prominent group among others. Furthermore, phenolics compound including flavonoid can be found in all plant tested. Additionally, according to [152], phenolics and many terpenoids are produced by mostly of all the plants.

Addition of alkali to some phenolics compound can be useful to detect the acidic compound such like anthocyanins. In other hand, colorless phenolics compound such like flavones, xanthones, flavonols, or hydroxycinnamic acid can be observed as yellow color by adding the alkalis. Ferric chloride solution also can be used to detect some phenolics compound while negative result is not necessarily proven that the phenolics are absent in the extract solution. Doing the screening test for phenolics compound in crude plant extract is quite give limited value due the presence of other substances in the mixture. Phytochemical screening only a rough test which are useful for guiding the further testing. Hence, for confirming the presence of phenolics compounds are better if used the chromatography technique [153].

Our result showed that triterpenes and steroid were found in almost all plant tested (**Table 11**). Triterpenes could be found in plant in a mixture with latex, resin, corks or waxes on the leaves. These compounds are functioning to herbivore deterrent. Production of this compound is known to be not dependence on the temperature change. This may be happened because of the molecule with higher number isoprene unit cause the plant allocate substrate to less costly isoprene [154]. Steroids in plants commonly occurred in combination with sugar to form the glycosides such as cardiac glycosides, steroidal saponins or glycoalkaloids[155].

Generally, terpenoids act as defense substance which play role in protection against herbivore and pathogen, allelochemical which may inhibit the growth of neighboring plants, as well as act as attractant for the seed dispersing animals. These

compounds also contribute in plant growth, development and reproduction. Some of compounds are acting as plant hormones [156].

In Asteraceae plants, sesquiterpene lactones and polyacetylenes are known to be the metabolites which fundamental to the evolutionary succession. Despite of that, diterpenes are known to be the most frequent metabolites as well [157]. Based on this research finding, diterpenes are found in almost all tested plants except *G. divaricata* **(Table 11)**. Diterpenes is known to be restricted in certain group and less frequent in the occurrence distribution compare to the other lower terpenoids. These compounds may be used as chemosystematic markers especially in Leguminosae family [158].

Based on this finding, among 16 tested plants, only 4 plants were showed the presence of saponin using the foam test **(Table 11).** Saponins are the glycosides which act as surfactant hence can dissolve in water forming the foam after shaking. These compounds are widely distributed in higher plants families. Saponins can be classified into two type including steroidal saponins and triterpenoid saponins. Steroidal saponins are known to be exclusively found in monocotyledon angiosperms whereas the triterpenoid saponins occur mostly in dicotyledon angiosperms. Some saponins were reported have antimalarial activity against *P. falciparum* [159].

Saponins are chemically complex structure composed of terpenoids and amphiphatic glycosides of steroids. These compounds consist of glycan (sugar chain) and aglycon called sapogenin. Saponins can be found in various parts of the plants however most plants store the saponins in the roots especially in secondary phloem and vascular cambium. This compound is mostly present in outer layer of the cells and may accumulate in phloem. Besides, saponin may be detected in leaves especially in palisade. However, leaves are more functioning to be the site of production rather than for storage function. Saponins may be disappeared when leaves are withered. The presence and concentration of saponins may be affected by various factors including abiotic and biotic stress which may lead the production as a defense chemical. Consequently, the saponin content may increase because of the hydrolysis of the stored precursors by the mechanism of the defense again pathogen. However, this compound has been known to

be played in innate immunity of the plant hence also may be presence in unchallenged plants [160].

Secondary metabolites are produced and store as a complex mixture and some compound groups are related to the other groups biosynthetically. Production of terpenoids was often accompanied by phenolics while alkaloids have more clustered distribution. Alkaloids usually can be found in certain and specific taxon hence this compound usually been used for chemotaxonomic marker. Production of other compounds such like sesquiterpene lactones, diterpenes, cardiac glycosides and iridoid glycosides also known to be restricted in certain plant [152]. Based on [30], alkaloid is commonly found in several plant families including Asteraceae.

The occurrence of alkaloids said to be scarce and the pyrolizidin alkaloids are known to be restricted only in Senecioneae and Eupatorieae tribes. Furthermore, alkaloids are known to be poorly evaluated in this family. Even though that this family is the largest consisting members (with 21,000 species from 1,100 genus), only 433 species from 92 plant's genus were reported containing the alkaloids [161]. Accordingly, alkaloids rarely been used as taxonomic markers for Asteraceae family.

Alkaloid known to be the waste product of plant metabolism, acted as growth regulator and reservoir storage of nitrogen. In nature, this compound is functioning as protective agents against the plant predators such as insects and herbivores [162]. Biosynthesis of alkaloids known to be gene-governed however, the production is affected by environmental condition. Environmental change can affect the concentration and amount of this compound through the impact on the growth and development of the plants. Commonly, alkaloids are produced in actively growing tissue (young tissue) hence environmental factors that affect growth such as light, temperature, soil moisture, latitude, supply of micronutrient (e.g. nitrogen, phosphorus, potassium) could affect the production of alkaloids. These factors might affect the biosynthesis or degradation hence can generate the lowering or increasing production from one to another species [163]. Alkaloids can be found in the roots, leaves, stem bark and seeds however commonly the woody part of plants posse low concentration [158].

Previous finding showed that nicotine production from seed *Nicotiana tabacum* known to be higher while the plant was germinated in the dark condition compared to the plant which was germinated in the environment with a sufficient amount of light. Respiration of the seed which was germinated in the dark might cause the losing of carbohydrate storage and they generate to use the amino acids from protein storage. Some amino acid which was mediated the alkaloids biosynthesis cannot be used for protein synthesis, therefore this compound is used for alkaloids production [163].

In this study, alkaloids test was performed using Dragendorff's test and Wagner's test. Based on the result of alkaloids screening using Dragendorff's and Wagner's reagent as shown in **Table 11**., some plants showed negative result tested with Dragendorff's while using Wagner's reagent were showed positive. It may be happened because each reagent may detect different type of alkaloids.

Dragendorff's reagent contained bismuth nitrate and potassium iodide which will generate the BiI_4- ion. The nitrogen atom in the structure could behave as a base which will react with acid through the acid base reaction [164]. Heavy metal in the reagent could react with amine group in the alkaloid's structure via coupling pair. The reaction will produce an insoluble precipitate which may give various color of precipitation depend on the type of alkaloid in the plants.

The result showed that the colors of precipitations are varies from yellowish, yellow, yellowish brown, orange, orange-red and brown-red. Dragendorff's reagent detect mostly for tertiary amine while the secondary amine will produce less color of precipitation. False positive result may be occurred due to presence of amine group in other compound such like protein. Hence, in this study, subsequent extraction to salting out the alkaloid was performed to prevent the false positive result. The extract solution was treated with ammonia to set the free amine and then followed by acid extraction to form the salts of alkaloids. The salts will react with the detecting reagent to produce the precipitate.

Wagner's reagent is able to detect some alkaloids with the presence of precipitation as well. Precipitation occurred when salt of alkaloid reacted with the acid (hydroiodide) from the formation of iodine and water consisted in the reagent. However,

some alkaloids such like caffeine, theobromine, piperine and urea are shown to be not precipitated at all after treating with this reagent. In other case, while strychnine gave satisfactory result when detected using this reagent, brucine is not. Some of precipitation showed to give up the portion of its iodine to the water. Some alkaloids which are soluble in water are become free as not as a salt from which cannot be detected with this reagent [165].

Secondary metabolites which play in defense mechanism of organism commonly produce in low concentration. The production of these compounds is an adaptive response to the environment or by the induction of stress condition. Plant secondary metabolites are synthesized in such restricted organ then transported to the different region of the tissue and organ hence these metabolites could be detected in the whole plant's cells. Transportation of this compound through the vascular tissue target to the storage site depend on the polarity of the compound. Hydrophobic compound such like terpenes could be stored in trichomes, resin ducts, cuticles or thylakoid membranes while the hydrophilic compounds (*e.g.* alkaloid, glucosinolates, tannins) could be stored in vacuole or idioblast. The accumulation of this metabolites is affected by many factors (*e.g*. drought, salinity, light, temperature, infection, interaction between species, *etc.*) then may be observed differently during the physiological change and developmental stage of the plants [166]. Alkaloid which known to be accumulated in the seed in the most plant could be happened because of these compounds is used for defense agent along with the utilization for nitrogen source during the seed germination. Additionally, production of flavonoid during the drought environment suggest their activity as radical scavenging [166]

Induction of environmental stressed such as light intensity, temperature, herbivore, microbial attack may trigger production of secondary metabolites as result the changes in genetic or protein level. In addition, the ability in production or certain classes of this compound is restricted to certain plant also. Many functional groups which construct the secondary metabolites complex structure may generate the various biological activities [151].

Antimalarial activity against 3D7 *P. falciparum* was performed using DNA fluorosence-based assay. The IC_{50} value of each ethanolic extract was used to classify the power of activity as shown in **Table 12**. According to this result, almost all tested plants which has been used for malaria treatment in some indigenous cultures showed antimalarial activity even though exhibited very weak-weak activity. In addition, 2 other plants (*B. pilosa* and *E. odoratum*) were inactive which showed the IC_{50} more than 100 µg/mL. This result suggested that the healing effect from these plants may be caused by other pharmacological properties which was related to curing other malarial symptoms which was not directly kill the parasites. In addition, the growing environment and method of preparation also become other factors. The non-scientific factor such like spirit, believe, and suggestion from the traditional healer might be the other cause of the healing properties from traditional medicine.

The best promising extract comes from the plants which commonly used by Ayuverdic medicine for fever treatment. Ethanolic extract of the aerial part *S. indicus* exhibited good-moderate activity against 3D7 *P. falciparum* with the IC_{50} 6.59 µg/mL. Another study which used the hexane and ethyl acetate extract of aerial part of the growing plant in other part of Thailand has been reported and showed has no activity against K1 *P. falciparum*. However, isolated sesquiterpene lactone eudesmanolides exhibited good-moderate antimalarial activity with the IC_{50} ranging from 2.32-6.47 µg/mL [129].

Clustered pattern of phytochemicals and antimalarial activity of 16 tested plants was observed descriptively as shown in **Fig 25**. Each species was classified into their tribal classification system. Classification system of Asteraceae family has been developed in recent decade by combining morphological and molecular data. Recent classification is refined by Panero and Funk in 2014 by using 10-14 chloroplast DNA (cpDNA) markers hence derived 12-13 major subfamilies (clade) [167]. Historically, the biggest change of Asteraceae classification has occurred in 1980s – 1980s by the molecular work done by Jansen et al. (1987, 1991 and 1996) [168].

As shown in **Fig 25**, the presence of phytochemicals showed were limited to the group of the compounds hence lead the difficulty to corelate between phylogeny and

clustered phytochemicals. Additionally, the presence of these phytochemicals can be affected by various factors including environmental of growing area hence the showed phytochemicals pattern might be changed if the source of collection is different.

Asteraceae has excellent morphologic and geographic diversity hence these plants able to produce a wide range of secondary metabolites including monoterpenes, sesquiterpenes, sesquiterpene lactones, diterpenes, triterpenes, polyacetylenes, phenolic acids, flavonoids, coumarins and benzofurans. Additionally, alkaloids are scarce in this family. However, pyrrolizidine alkaloids can be found and become the typical compound in the tribe of Senecioneae and Eupatorieae. In Eupatoriaeae tribe, the pyrrolizidine alkaloids are found to be lesser extent than Senecioneae. [169, 170].

In this family, flavonoids are the most occurrence compound followed by polyacetylene, sesquiterpenes, monoterpenes, diterpenes, coumarins, triterpenes and benzofurans respectively. Although triterpenes are known to be less abundance, however, they occur in 28 of 35 tribes of Asteraceae. These compounds act as a plant's defense, plant-plant interaction and plant-insect interaction. The other terpenes such as sesquiterpenes (including sesquiterpene lactones) and monoterpenes are equally abundant in Asteraceae [169].

Senecioneae is known to be a sister clade with all the other tribes of Asteroideae subfamily including Anthemideae, Inuleae and Heliantheae alliance [169]. ITS marker has been refined the relationship on inter and intra-generic level in this tribe especially for *Senecio* [171]. The notable phytochemical in this tribe is pyrrolizidine alkaloids which are derived from amino acid ornithine. These phytochemicals are toxic and act as deterrent to protect them from the herbivore. Eremophilanes sesquiterpene lactones also become the excellent marker of this tribe hence useful as sub-tribal and inter-generic classification. In other hand, polyacetylenes and coumarins which are commonly occurred in this family, known to be absent in this tribe [171]. In Vernonieae tribe, the bitter tasting compound, sesquiterpene lactones also been used in the tribal systematics. Typical SLs occurred in this tribe including glaucolides, guaianolides, germacranolides, hirsutinoldes, eremanolides, furoheliangolides, elemanolides along with nerolidol derivatives as well as non-SLs coumarins [169]. Flavonoids, sesquiterpene lactones and

polyacetylenes are the three major compounds in Anthemideae tribe. SLs are known to be taxonomic marker for this tribe [169]. Tribe Inuleae can be divided into two groups which are Inuleae-Inuleae and Inuleae-Plucheinae. The tribe Inuleae-inuleae including *Blumea* genus while the Inuleae-Plucheinae including *Sphaeranthus* genus. Typical chemicals in this tribe is oligosaccharide inuline while the SLs 8,12 eudesmanolides known to be predominant in Inuleae along with their sister group Heliantheae. Benzofuran or benzopyran which become diagnostic character of Asteroideae can be found in several Inuleae-Inuleae however not present in Inuleae-Plucheineae [169]. Diterpenes are considered as the frequent compounds occurred in Asteraceae. Based on chemicals occurrence investigation in this family, diterpenes are most concentrated in subfamily Asteroideae compared to the other subfamilies. Occurrences of this compounds are varies among the tribes [157, 172]. Based on the finding, diterpenes are detected in almost of all investigated plants except *G. divaricata.* This plant belong to Senecioneae tribe which known to be the largest tribe in Asteroideae sub-family. However, based on chemicals mapping of diterpenes, this tribe showed to have the lowest occurrence of diterpenes (28/3200 species) [172].

Based on the result as shown in **fig 25**., our result showed that among 16 tested plants, 8 plants species showed antimalarial activity which were exhibited very weak till good-moderate. Scattered pattern of the power of antimalarial activity has occurred in the phylogeny. However, the result showed that in tested plants from Heliantheae alliance exhibited very weak- inactive antimalarial activity. However, due the limited number of the tested plants and the un-even number of member group of each tribe, this result is not enough as a base for judgement to jump into the conclusion to clustering the power of bioactivity based on the tribal level. In addition, the part used of the plants for testing were different, hence may lead inconsistency when changing the part of the plant tested.

In order to investigate the sharing bioactivity between closed related species with the artemisinin producing species (*A. annua*), we investigated four taxa of *Artemisia* including *A. dracunculus, A. chinensis, A. lactiflora* and *A. vulgaris.* Based on the result, *A. vulgaris* and *A. chinensis* showed weak antimalarial activity. In other hand, *A. lactiflora* showed inactive even has a more closed relatedness with *A. vulgaris*. Besides,

A. dracunculus which showed to be not in the similar clade with the three other *Artemisia* species also exhibited inactive antimalarial activity.

Using phylogeny bioprospecting, we discovered new medicinal activity of the plants which was not used as herbal medicine for any treatment for malaria and its associated symptoms. In South China, the whole plant of *A. chinensis* is used for treatment of diabetes, cold, furuncle, and carbuncle [173]. *A. chinensis* was chosen due to the close relatedness with *A. annua* and showed the inhibition against 3D7 *P. falciparum* with the IC_{50} 18.30 µg/mL and was considered as a weak activity. According to [9][174, 175], secondary metabolites can be gene-governed due to their function as defense mechanism hence will be maintained during evolution. Hence, similar bioactivity may be shared between closed related species.

The antimalarial activity of *Artemisia* species was assumed caused by the effect of artemisinin or along with other antimalarial active compound consisted in the extract which may act agonist or antagonist. Therefore, the power of antimalarial activity may different. *A. vulgaris* and *A. dracunculus* were reported contained artemisinin in a lower concentration than *A. annua* [176].

Our study supported that the phylogeny is useful to help narrow down the selection for targeting the promising clade for investigating the antimalarial activity. However, the un-even numbers of each tribal give a difficulty to observe the clustered pattern of bioactivity. The association between phylogeny and bioactivity could not be assessed due to the unbalance of number of taxa in each tribe (e.g. 4 *Artemisia* species were used in this study and there was no other species in Anthemideae tribe). Additionally, the similar part of investigated plant should be used to generate the comparable result of activity. Single group of compounds which was typical in each tribe may be selected for further study in order to know the clustered pattern rather than use the group of compounds.

Validation of antimalarial activity through doing different type of antimalarial assay should be performed to get the robustness of the activity result. The different antimalarial assay might give different power of activity result hence might generate different clusterred pattern result in phylogenetic tree. According to our finding, Inuleae

and Anthemideae tribe should be further investigated for discovering the antimalarial plants. The antimalarial activity should be performed in chloroquine resistant strain as well. In addition, the plants which showed the good-moderate till weak activity is worth to further be investigated to find which active compound was responsible for the activity.This approach may be used for another disease for selecting the candidate group of taxa to minimize the expenditure and time in antimalarial plant-based drug discovery.

CONCLUSSION

Clustered pattern was shown in phylogenetic tree of ethnobotanical medicinal plants for malaria majorly in Asteraceae family along with fever treatment. Other highlighted families including Apocynaceae, Rubiaceae and Euphorbiaceae were consistently found to be clustered for malaria treatment. In other hand, plants for fever treatment also clumped in Cucurbitacaeae. These highlighted families may be required for further experiment to investigate the antimalarial activity.

Among 16 selected Asteraceae medicinal plants, 1 ethanolic extract showed good-moderate 4 ethanolic extract showed weak antimalarial activity, 3 ethanolic extract showed very weak and the rest is inactive. The best active ethanolic extract was shown by ethanolic extract from aerial part *S. indicus.* New medicinal property as antimalarial of *A. chinensis* which never been used as treatment in traditional medicine was discovered by using this approach.

Phylogeny approach is useful to narrow down the selection of candidate taxa for screening antimalarial activity on ethnobotanical data in order to minimize the expenditure and time in laboratory experiment.

REFERENCES

1. WHO, *World Malaria Report 2019*. 2019: World Health Organization 2019.
2. Adebayo, J. and A. Krettli, *Potential antimalarials from Nigerian plants: a review.* Journal of Ethnopharmacology, 2011. **133**(2): p. 289-302.
3. Shankar, R., S. Deb, and B.K. Sharma, *Antimalarial plants of northeast India: An overview.* Journal of Ayurveda and integrative medicine, 2012. **3**(1): p. 10-16.
4. Evans, W.C., *Trease and Evans : Pharmacognosy*. 2009, China: Saunders Elsevier. 614.
5. Meshnick, S.R., *Artemisinin: mechanisms of action, resistance and toxicity.* International Journal for Parasitology, 2002. **32**(13): p. 1655-1660.
6. Manel, O., et al., *Plasmodium falciparum resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination.* J Parasite, 2018. **25**.
7. Sinha, S., et al., *Development in Assay Methods for in Vitro Antimalarial Drug Efficacy Testing: A Systematic Review.* Frontiers in pharmacology, 2017. **8**: p. 754.
8. Calixto, J., *Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents).* Braz J Med Biol Res, 2000. **33**(2): p. 179-189.
9. Haris, S.-L.C., et al., *Phylogenies reveal predictive power of traditional medicine in bioprospecting.* J Proceedings of the National Academy of Sciences, 2012. **109**(39): p. 15835-15840.
10. Saslis-Lagoudakis, C.H., et al., *The use of phylogeny to interpret cross-cultural patterns in plant use and guide medicinal plant discovery: an example from Pterocarpus (Leguminosae).* PLOS ONE, 2011. **6**(7): p. e22275.
11. Shaw, A.J., et al., *Phylogeography and phylodemography.* Bryologist, 2002: p. 373-383.
12. Liu, H., et al., *Molecular authentication and differentiation of Dendrobium species by rDNA ITS region sequence analysis.* AMB Express, 2019. **9**(1): p. 53.
13. Cheng, T., et al., *Barcoding the kingdom Plantae: new PCR primers for ITS regions of plants with improved universality and specificity.* Molecular Ecology Resources, 2016. **16**(1): p. 138-149.
14. Linder, C.R., L.A. Moore, and R.B. Jackson, *A universal molecular method for identifying*

underground plant parts to species. Mol Ecol, 2000. **9**(10): p. 1549-59.

15. Ivanescu, B., A. Miron, and A. Corciova, *Sesquiterpene lactones from Artemisia genus: biological activities and methods of analysis.* Journal of analytical methods in chemistry, 2015. **2015**.
16. Deans, S.G. and E.J.M. Simpson, *HERBS | Herbs of the Compositae*, in *Encyclopedia of Food Sciences and Nutrition (Second Edition)*, B. Caballero, Editor. 2003, Academic Press: Oxford. p. 3077-3081.
17. Haldar, K. and N. Mohandas, *Malaria, erythrocytic infection, and anemia.* Hematology. American Society of Hematology. Education Program, 2009: p. 87-93.
18. Maier, A.G., et al., *Plasmodium falciparum.* Trends in Parasitology, 2019. **35**(6): p. 481-482.
19. Griffith, K.S., et al., *Treatment of malaria in the United States: a systematic review.* Jama, 2007. **297**(20): p. 2264-2277.
20. Prevention, C.f.D.C.a. *Malaria*. 2017.
21. Poostchi, M., et al., *Image analysis and machine learning for detecting malaria.* Translational Research, 2018. **194**.
22. Tangpukdee, N., et al., *Malaria diagnosis: a brief review.* Korean Journal of Parasitology, 2009. **47**(2): p. 93.
23. Meshnick, S.R. and M.J. Dobson, *The history of antimalarial drugs*, in *Antimalarial chemotherapy*. 2001, Springer. p. 15-25.
24. Wang, J., et al., *Artemisinin, the Magic Drug Discovered from Traditional Chinese Medicine.* Engineering, 2019. **5**(1): p. 32-39.
25. Ferreira, J.F. and D.L. Luthria, *Drying affects artemisinin, dihydroartemisinic acid, artemisinic acid, and the antioxidant capacity of Artemisia annua L. leaves.* Journal of Agricultural and Food Chemistry, 2010. **58**(3): p. 1691-1698.
26. Duru, V., B. Witkowski, and D. Ménard, *Plasmodium falciparum resistance to artemisinin derivatives and piperaquine: a major challenge for malaria elimination in Cambodia.* The American Journal of Tropical Medicine Hygiene, 2016. **95**(6): p. 1228-1238.
27. Lu, F., et al., *A brief history of artemisinin: Modes of action and mechanisms of resistance.* Chinese Journal of Natural Medicines, 2019. **17**(5): p. 331-336.

28. Suresh, N. and K. Haldar, *Mechanisms of artemisinin resistance in Plasmodium falciparum malaria.* Current Opinion in Pharmacology, 2018. **42**: p. 46-54.

29. Waksmundzka-Hajnos, M., J. Sherma, and T. Kowalska, *Thin Layer Chromatography in Phytochemistry*. 2008, US: Taylor & Francis Group, LLC. 888.

30. Shah, B. and A.K. Seth, *Textbook of Pharmacognosy and Phytochemistry : First edition*. 2010, India: Elsevier.

31. Kumar, G.S. and K.N. Jayaveera, *A Textbook of Pharmacognosy and Phytochemistry*. 2014, New Delhi: S.Chand & Company PVT.LTD. 288.

32. Soto-Hernandez, M., M. Palma-Tenango, and M.d.R. Garcia-Mateos, *Phenolic Compounds : Natural Sources, Importance and Applications*. 2017, Croatia: InTech.

33. Lattanzio, V., *Phenolic Compounds: Introduction*. 2013. p. 1543-1580.

34. Laura, A., et al., *Phenolic compounds*, in *Postharvest physiology and biochemistry of fruits and vegetables*. 2019, Elsevier. p. 253-271.

35. Ivanescu, B., A. Miron, and A. Corciova, *Sesquiterpene Lactones from Artemisia Genus: Biological Activities and Methods of Analysis.* Journal of analytical methods in chemistry, 2015. **2015**: p. 247685-247685.

36. Ghantous, A., et al., *What made sesquiterpene lactones reach cancer clinical trials? Drug Discov Today.* Drug discovery today, 2010. **15**: p. 668-78.

37. Chadwick, M., et al., *Sesquiterpenoids lactones: benefits to plants and people.* International journal of molecular sciences, 2013. **14**(6): p. 12780-12805.

38. Mateji**ć**, J., Z. Šarac, and V. Ran**đ**elovi**ć**, *Pharmacological activity of sesquiterpene lactones.* Biotechnology Biotechnological Equipment, 2010. **24**(sup1): p. 95-100.

39. Pedersen, M.M., et al., *Antimalarial sesquiterpene lactones from Distephanus angulifolius.* Phytochemistry, 2009. **70**(5): p. 601-607.

40. Rukunga, G., et al., *Anti-plasmodial activity of the extracts and two sesquiterpenes from Cyperus articulatus.* Fitoterapia, 2008. **79**(3): p. 188-190.

41. François, G. and C.M. Passreiter, *Pseudoguaianolide sesquiterpene lactones with high activities against the human malaria parasite Plasmodium falciparum.* Phytother Res, 2004. **18**(2): p. 184-186.

42. Sarker, S.D., Z. Latif, and A.I. Gray, *Method in Biotechnology : natural products isolation*

second edition. Humana Press Inc, 2006: p. 1-515.

43. Rates, S.M.K., *Plants as source of drugs.* Toxicon, 2001. **39**(5): p. 603-613.

44. Rocha, R. and E. Melo, *Influence of drying process on the quality of medicinal plants: A review.* Journal of Medicinal Plants Research, 2011. **5**(33): p. 7076-7084.

45. Zhang, Q.-W., L.-G. Lin, and W.-C. Ye, *Techniques for extraction and isolation of natural products: a comprehensive review.* Chinese medicine, 2018. **13**: p. 20-20.

46. Ramluckan, K., K. Moodley, and F. Bux, *An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the Soxhlet extraction method.* Fuel, 2013. **116**: p. 103-108.

47. Pandey, A. and S. Tripathi, *Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug.* Journal of Pharmacognosy Phytochemistry, 2014. **2**(5).

48. ICH, *Impurities : guideline for residual solvents QRC(R6)*, I.C.f.H.o.T.R.f.P.f.H. Use, Editor. 2016.

49. Zhang, Q.-W., L.-G. Lin, and W.-C. Ye, *Techniques for extraction and isolation of natural products: a comprehensive review.* Chinese Medicine, 2018. **13**(1): p. 20.

50. Rasul, M.G., *Extraction, Isolation and Characterization of Natural Products from Medicinal Plants.* 2011. **2**(6): p. 1-6.

51. Kaur, H., et al., *Antiplasmodial medicinal plants: a literature review on efficacy, selectivity and phytochemistry of crude plant extracts.* Journal of Biologically Active Products from Nature, 2018. **8**(5): p. 272-294.

52. Reddy, B.N., *Basics for the construction of phylogenetic trees.* 2011.

53. Abiri, R., et al., *Towards a better understanding of Artemisia vulgaris: Botany, phytochemistry, pharmacological and biotechnological potential.* Food Res Int, 2018. **109**: p. 403-415.

54. Zeb, S., et al., *Pharmacology, Taxonomy and Phytochemistry of the genus Artemisia specifically from Pakistan: a comprehensive review.* J Pharmaceutical Biomedical Research, 2019.

55. Milliken, W., *Plants for malaria.* 1997.

56. Le Tran, Q., et al., *In vitro antiplasmodial activity of antimalarial medicinal plants used in*

Vietnamese traditional medicine. Journal of Ethnopharmacology, 2003. **86**(2-3): p. 249-252.

57. Ramazani, A., et al., *In vitro antiplasmodial and phytochemical study of five Artemisia species from Iran and in vivo activity of two species.* Parasitol Res, 2010. **107**(3): p. 593-599.

58. Thiengsusuk, A., W. Chaijaroenkul, and K. Na-Bangchang, *Antimalarial activities of medicinal plants and herbal formulations used in Thai traditional medicine.* Parasitol Res, 2013. **112**(4): p. 1475-1481.

59. Nigam, M., et al., *Bioactive Compounds and Health Benefits of Artemisia Species.* Natural Product Communications, 2019. **14**(7): p. 1934578X19850354.

60. Mannan, A., et al., *Survey of artemisinin production by diverse Artemisia species in northern Pakistan.* Malaria journal, 2010. **9**(1): p. 310.

61. Bora, K.S. and A. Sharma, *The genus Artemisia: a comprehensive review.* Pharmaceutical Biology, 2011. **49**(1): p. 101-109.

62. Hussain, A., et al., *Pharmacological promises of genus Artemisia (Asteraceae): A Review.* B Life Environ. Sci, 2017. **54**: p. 265-287.

63. Amidon, C., M.A. Thomas, and E. Kennel, *Artemisia : an essential guide from the herb society of america*. 2014: The Herbs Society of America

64. Xiao, M.-t., et al., *A novel polyacetylene from the aerial parts of Artemisia lactiflora.* Phytochemistry Letters, 2014. **8**: p. 52-54.

65. Li, D.-l., et al., *Ethnobotanical survey of herbal tea plants from the traditional markets in Chaoshan, China.* Journal of Ethnopharmacology, 2017. **205**: p. 195-206.

66. Kulprachakarn, K., et al., *Antioxidant Properties and Cytotoxicity of White Mugwort (Artemisia lactiflora) Leaf Extract in Human Hepatocellular Carcinoma Cell Line.* Walailak Journal of Science, 2019. **16**(3): p. 185-192.

67. Bhuiyan, M.N.I., J.U. Chowdhury, and J. Begum, *Chemical components in volatile oil from Blumea balsamifera (L.) DC.* Bangladesh Journal of Botany, 2009. **38**(1): p. 107-109.

68. Pang, Y., et al., *Blumea balsamifera--a phytochemical and pharmacological review.* Molecules, 2014. **19**(7): p. 9453-77.

69. Noor Rain, A., et al., *Antiplasmodial properties of some Malaysian medicinal plants.* J Trop

Biomed, 2007. **24**(1): p. 29-35.

70. Xuan, N. and Dung, *Selection of traditional medicines for study.* Journal of Ethnopharmacology, 1991. **32**(1): p. 57-70.

71. Abdillah, S., et al., *Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia.* Asian Pacific Journal of Tropical Disease, 2015. **5**(6): p. 454-457.

72. Chauhan, A. and S. Rijhwani, *A Comprehensive Review on Phytochemistry of Ageratum conyzoides linn.(Goat weed).* International Journal of Engineering Technology Management, 2015. **3**.

73. Joshi, B., et al., *In vitro antileishmanial and antimalarial activity of selected plants of Nepal.* J Intercult Ethnopharmacol, 2016. **5**(4): p. 383-389.

74. de Madureira, M.d.C., et al., *Antimalarial activity of medicinal plants used in traditional medicine in S. Tomé and Prıncipe islands.* Journal of Ethnopharmacology, 2002. **81**(1): p. 23-29.

75. Kaur, R. and N. Dogra, *A review on traditional uses, chemical constituents and pharmacology of Ageratum conyzoides L.(Asteraceae).* Int J Pharmaceutical Biol Arch, 2014. **5**(5): p. 33-45.

76. Grombone-Guaratini, M.T., V.N. Solferini, and J. Semir, *Reproductive biology in species of Bidens L.(Asteraceae).* Scientia Agricola, 2004. **61**(2): p. 185-189.

77. Bartolome, A.P., I.M. Villaseñor, and W.-C. Yang, *Bidens pilosa L. (Asteraceae): botanical properties, traditional uses, phytochemistry, and pharmacology.* J Evidence-based Complementary Alternative Medicine, 2013. **2013**.

78. Andrade‐Neto, V.F., et al., *Antimalarial activity of Bidens pilosa L.(Asteraceae) ethanol extracts from wild plants collected in various localities or plants cultivated in humus soil.* J Phytotheraphy Research, 2004. **18**(8): p. 634-639.

79. Titanji, V.P., D. Zofou, and M.N. Ngemenya, *The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine.* African Journal of Traditional, Complementary and Alternative Medicines, 2008. **5**(3): p. 302.

80. Rajamurugan, R., et al., *Identification, quantification of bioactive constituents, evaluation of antioxidant and in vivo acute toxicity property from the methanol extract of Vernonia*

cinerea leaf extract. Pharmaceutical Biology, 2011. **49**(12): p. 1311-1320.

81. Shelar, D., S. Tikole, and T. Kakade, *Vernonia cinerea: a review.* Current Pharma Research, 2014. **4**(3): p. 1194.

82. Guha, G., et al., *Therapeutic Potential of Polar and Non-Polar Extracts of Cyanthillium cinereum In Vitro.* J Evidence-based Complementary Alternative Medicine, 2011. **2011**.

83. Chea, A., et al., *Antimalarial activity of sesquiterpene lactones from Vernonia cinerea.* Chem Pharm Bull (Tokyo), 2006. **54**(10): p. 1437-9.

84. Dogra, N.K. and S. Kumar, *A review on ethno-medicinal uses and pharmacology of Vernonia cinerea Less.* J Natural Product Research, 2015. **29**(12): p. 1102-1117.

85. Soma, A., et al., *Antiplasmodial activity of Vernonia cinerea Less (Asteraceae), a plant used in traditional medicine in Burkina Faso to treat malaria.* African Journal of Pharmacy Pharmacology, 2017. **11**(5): p. 87-93.

86. Verma, S., *Phytochemical and pharmacological investigation of Vernonia cinerea: Asteraceae.* Pharma Innovat J, 2018. **7**: p. 519-521.

87. Vanijajiva, O. and N. Kundee, *Genetic uniformity of exotic medicinal plant Gynura divaricata in Thailand.* Research Journal of Phranakhon Rajabhat: Science Technology, 2014. **9**(1): p. 77-86.

88. Poeaim, S. and O. Vanijajiva. *Morphological Identification and In Vitro Cytotoxic Evaluation of Crude Extracts from Jak-Na-Rai (Gynura divaricata (L.) DC.)*. in *16th Asian Agricultural Conference*. 2010.

89. Vanijajiva, O. and J.W. Kadereit, *A revision of Gynura (Asteraceae: Senecioneae).* Journal of Systematics Evolution, 2011. **49**(4): p. 285-314.

90. Chen, J., et al., *Detection and toxicity evaluation of pyrrolizidine alkaloids in medicinal plants gynura bicolor and gynura divaricata collected from different Chinese locations*. Chemistry Biodiversity, 2017. **14**(2): p. e1600221.

91. Xu, B.-Q. and Y.-Q. Zhang, *Bioactive components of Gynura divaricata and its potential use in health, food and medicine: a mini-review.* Africa Journal of Traditional, Complementary Alternative Medicines, 2017. **14**(3): p. 113-127.

92. Chen, J., et al., *Investigation of the volatile constituents of different Gynura species from two Chinese origins by SPME/GC-MS.* Natural Product Communications, 2012. **7**(5): p.

1934578X1200700528.

93. Siriwatanametanon, N. and M. Heinrich, *The Thai Medicinal Plant Gynura Pseudochina var. hispida: Chemical Composition and in vitro NF-ΚB Inhibitory Activity.* Natural Product Communications, 2011. **6**(5): p. 1934578X1100600512.

94. Siriwatanametanon, N., et al., *Traditionally used Thai medicinal plants: in vitro anti-inflammatory, anticancer and antioxidant activities.* J Ethnopharmacol, 2010. **130**(2): p. 196-207.

95. Moelyono Moektiwardoyo, W., et al., *The Potential of Dewa Leaves (Gynura Pseudochina (L) D.C) and Temu Ireng Rhizomes (Curcuma aeruginosa Roxb.) as Medicinal Herbs for Dengue Fever Treatment.* Procedia Chemistry, 2014. **13**: p. 134-141.

96. Kuldeep, G. and A. Pathak, *Pharmacognostic and Phytochemical Evaluation of Tridax procumbens Linn.* Journal of Pharmacognosy Phytochemistry, 2013. **1**(5).

97. Pandey, A. and S. Tripathi, *A Review on Pharmacognosy, Pre-phytochemistry and Pharmacological analysis of Tridax procumbens.* PharmaTutor, 2014. **2**(4): p. 78-86.

98. Appiah-Opong, R., et al., *Antiplasmodial activity of extracts of Tridax procumbens and Phyllanthus amarus in in vitro Plasmodium falciparum culture systems.* Ghana Medical Journal, 2011. **45**(4).

99. Cáceres, A., et al., *Plants used in Guatemala for the treatment of protozoal infections. I. Screening of activity to bacteria, fungi and American trypanosomes of 13 native plants.* Journal of Ethnopharmacology, 1998. **62**(3): p. 195-202.

100. Upadhyay, B., A.K. Dhaker, and A. Kumar, *Ethnomedicinal and ethnopharmaco-statistical studies of Eastern Rajasthan, India.* Journal of Ethnopharmacology, 2010. **129**(1): p. 64-86.

101. Kaushik, N.K., et al., *Evaluation of antiplasmodial activity of medicinal plants from North Indian Buchpora and South Indian Eastern Ghats.* Malaria journal, 2015. **14**(1): p. 65.

102. Muthaura, C., et al., *Antiplasmodial potential of traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast.* Journal of Ethnopharmacology, 2015. **170**: p. 148-157.

103. Christudas, S., T.M. Kulathivel, and P. Agastian, *Phytochemical and antibacterial studies of leaves of Tridax procumbens L.* Asian Pacific Journal of Tropical Biomedicine, 2012. **2**(1, Supplement): p. S159-S161.

104. Andriana, Y., et al., *Antihyperuricemia, Antioxidant, and Antibacterial Activities of Tridax procumbens L.* Foods, 2019. **8**(1).

105. DSNBK, P. and L. Rao, *Evaluation of Pharmacognostic, Phytochemical and Physicochemical Standards of Wedelia Trilobata (L.) Root.*

106. Hui, Y., et al., *Eudesmanolides and Other Constituents from the Flowers of Wedelia trilobata.* Chemistry Biodiversity, 2018. **15**(3): p. 1700411.

107. Fauziana, M. and R. Susandarini, *Species Diversity and Potential Use of Asteraceae in Tawangmangu, Karanganyar Regency, Central Java.* Journal of Tropical Biodiversity Biotechnology, 2019. **4**(1): p. 18-23.

108. Ton That, Q., et al., *Wedelolides A and B: Novel Sesquiterpene δ-Lactones, (9R)-Eudesman-9,12-olides, from Wedelia trilobata.* The Journal of Organic Chemistry, 2007. **72**(19): p. 7102-7105.

109. Zahara, M. *Description of Chromolaena odorata LRM King and H. Robinson as medicinal plant: A Review*. in *IOP Conference Series: Materials Science and Engineering*. 2019. IOP Publishing.

110. Fasola, T.R. and P.C. Iyamah, *Comparing the phytochemical composition of some plant parts commonly used in the treatment of malaria.* International Journal of Pure Applied Sciences Technology, 2014. **21**(1): p. 1.

111. Ezenyi, I.C., et al., *Antiplasmodial activity-aided isolation and identification of quercetin-4'-methyl ether in Chromolaena odorata leaf fraction with high activity against chloroquine-resistant Plasmodium falciparum.* Parasitol Res, 2014. **113**(12): p. 4415-4422.

112. Félicien, A., et al., *Chemical composition and Biological activities of the Essential oil extracted from the Fresh leaves of Chromolaena odorata (L. Robinson) growing in Benin.* ISCA Journal of Biological Sciences, 2012. **1**(3): p. 7-13.

113. Hassanzadeh, M.K., et al., *Tarragon (Artemisia dracunculus L.) Oils*, in *Essential Oils in Food Preservation, Flavor and Safety*. 2016, Elsevier. p. 813-817.

114. Obolskiy, D., et al., *Artemisia dracunculus L. (Tarragon): A Critical Review of Its Traditional Use, Chemical Composition, Pharmacology, and Safety.* Journal of Agricultural and Food Chemistry, 2011. **59**(21): p. 11367-11384.

115. Eidi, A., et al., *Antinociceptive and anti-inflammatory effects of the aerial parts of*

Artemisia dracunculus in mice. Pharmaceutical Biology, 2015. **54**.

116. Lopes-Lutz, D., et al., *Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils.* Phytochemistry, 2008. **69**(8): p. 1732-1738.

117. Tabanca, N., et al., *Eupatorium capillifolium essential oil: chemical composition, antifungal activity, and insecticidal activity.* J natural Product Communications, 2010. **5**(9): p. 1934578X1000500913.

118. Johnson, M.F., *Eupatorieae (Asteraceae) in Virginia: Eupatorium L.* Castanea, 1974. **39**(3): p. 205-228.

119. Uehara, A., et al., *Further characterization of foliar flavonoids in Crossostephium chinense and their geographic variation.* Natural Product Communications, 2014. **9**(2): p. 1934578X1400900207.

120. Thomson, A.T., *The London Dispensatory : A practical synopsis of materia medica, pharmacy and therapeutics*. 1830, London: London.

121. Wiart, C., *Anti-Inflammatory Plants*, in *Ethnopharmacology of Medicinal Plants: Asia and the Pacific*. 2007, Humana Press: Totowa, NJ. p. 1-55.

122. Li, T.S.C., *Taiwanese Native Medicinal Plants : Phytopharmacology and Therapeutic Values* CRC Press Taylor & Francis Group, LLC.

123. Wu, Q., et al., *Novel sesquiterpene and coumarin constituents from the whole herbs of Crossostephium chinense.* Journal of Asian Natural Products Research, 2009. **11**(1): p. 85-90.

124. Uehara, A., et al., *Further characterization of foliar flavonoids in Crossostephium chinense and their geographic variation.* Nat Prod Commun, 2014. **9**(2): p. 163-4.

125. Chang, T.-N., et al., *Hepatoprotective effect of Crossostephium chinensis (L.) makino in rats.* The American Journal of Chinese Medicine, 2011. **39**(03): p. 503-521.

126. Chakraborty, A., et al., *Sphaeranthus indicus Linn.: A phytopharmacological review.* Int J Drug Formul Res, 2010. **1**: p. 113-33.

127. Ramachandran, S., *Review on Sphaeranthus indicus Linn.(Koṭṭaikkarantai).* Pharmacognosy reviews, 2013. **7**(14): p. 157.

128. Galani, V.J., B.G. Patel, and D.G. Rana, *Sphaeranthus indicus Linn.: A phytopharmacological review.* Int J Ayurveda Res, 2010. **1**(4): p. 247-53.

129. Sangsopha, W., et al., *Cytotoxic and antimalarial constituents from aerial parts of Sphaeranthus indicus.* Phytochemistry Letters, 2016. **17**: p. 278-281.

130. Dubey, S., et al., *Phytochemistry, pharmacology and toxicology of Spilanthes acmella: a review.* Advances in Pharmacological Sciences, 2013. **2013**.

131. Joseph, B., J. George, and M.J.W.J.P.R. Jeevitha, *The role of Acmella Olerecea in Medicine—A review.* World Journal of Pharmaceutical Research, 2017. **2**: p. 2781-2792.

132. Spelman, K., et al., *The traditional medicine Spilanthes acmella, and the alkylamides spilanthol and undeca‐2e‐ene‐8, 10‐diynoic acid isobutylamide, demonstrate in vitro and in vivo antimalarial activity.* Phytotheraphy Research, 2011. **25**(7): p. 1098-1101.

133. Abeysiri, G.R.P.I., et al., *Screening of phytochemical, physico-chemical and bioactivity of different parts of Acmella oleraceae Murr. (Asteraceae), a natural remedy for toothache.* Industrial Crops and Products, 2013. **50**: p. 852-856.

134. Aid, A.N. and M. Jeanmaire, *The ethnobotany of psychoactive plant use: a phylogenetic perspective.* PeerJ, 2016. **4**: p. e2546-e2546.

135. Alebiosu, C. and A. Yusuf, *Phytochemical screening, thin-layer chromatographic studies and UV analysis of extracts of Citrullus lanatus.* J Pharm Chem Biol Sci, 2015. **3**(2): p. 214-220.

136. Coronado-Aceves, E.W., et al., *Reynosin and santamarine: two sesquiterpene lactones from Ambrosia confertiflora with bactericidal activity against clinical strains of Mycobacterium tuberculosis.* Pharmaceutical Biology, 2016. **54**(11): p. 2623-2628.

137. Prasad, M.A., C.P. Zolnik, and J. Molina, *Leveraging phytochemicals: the plant phylogeny predicts sources of novel antibacterial compounds.* Future Science OA, 2019. **5**(7): p. FSO407.

138. Manissorn, J., *Molecular Analysis of Phyllanthus spp. in Thailand based on RAPD and DNA sequencing*, in *Pharmacognosy and Pharmaceutical Botany*. 2010, Chulalongkorn University: Faculty of Pharmaceutical Sciences.

139. Katoh, K. and D.M. Standley, *MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability.* Molecular Biology and Evolution, 2013. **30**(4): p. 772-780.

140. Chowdhury, B. and G. Garai, *A review on multiple sequence alignment from the perspective*

of genetic algorithm. Genomics, 2017. **109**(5): p. 419-431.

141. Katoh, K., et al., *MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform.* Nucleic Acids Research, 2002. **30**(14): p. 3059-3066.

142. Goertzen, L.R., et al., *ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny of the Asteraceae.* Molecular Phylogenetics and Evolution, 2003. **29**(2): p. 216-234.

143. McDonald‐Spicer, C., et al., *Big data for a large clade: Bioregionalization and ancestral range estimation in the daisy family (Asteraceae).* Journal of Biogeography, 2019. **46**(2): p. 255-267.

144. Mandel, J.R., et al., *The Compositae tree of life in the age of phylogenomics.* Journal of Systematics Evolution, 2017. **55**(4): p. 405-410.

145. Gallon, M.E., et al., *Metabolomic analysis applied to chemosystematics and evolution of megadiverse Brazilian Vernonieae (Asteraceae).* Phytochemistry, 2018. **150**: p. 93-105.

146. Moujir, L., et al., *Applications of sesquiterpene lactones: a review of some potential success cases.* Applied Sciences, 2020. **10**(9): p. 3001.

147. M., O.N.P., B.V. E., and W.S. A., *The molecular mechanism of action of artemisinin--the debate continues.* Molecules (Basel, Switzerland), 2010. **15**(3): p. 1705-1721.

148. Gunjan, S., et al., *Artemisinin derivatives and synthetic trioxane trigger apoptotic cell death in asexual stages of plasmodium.* Frontiers in Cellular Infection Microbiology, 2018. **8**: p. 256.

149. S., S., *The Chemistry of Natural Products: 6: Plenary Lectures Presented at the Sixth International Symposium on the Chemistry of Natural Products*. 2013: Elsevier Science.

150. C., S.F., *Sesquiterpene lactones as taxonomic characters in the asteraceae.* The Botanical Review, 1982. **48**(2): p. 121-594.

151. Ncube, B., J.F. Finnie, and J. Van Staden, *Quality from the field: The impact of environmental factors as quality determinants in medicinal plants.* South African Journal of Botany, 2012. **82**: p. 11-20.

152. Wink, M., *Plant secondary metabolism: diversity, function and its evolution.* Natural Product Communications, 2008. **3**(8): p. 1934578X0800300801.

153. Harborne, J.B., *Methods in plant biochemistry. Volume 1. Plant phenolics*. 1989: Academic

Press Ltd.

154. Holopainen, J., et al., *Ecological Functions of Terpenoids in Changing Climates*. 2013. p. 2913-2940.

155. Heftmann, E., *Biochemistry of plant steroids.* Journal Annual Review of Plant Physiology, 1963. **14**(1): p. 225-248.

156. Ashour, M., M. Wink, and J. Gershenzon, *Biochemistry of terpenoids: monoterpenes, sesquiterpenes and diterpenes.* Annual Plant Reviews Online, 2018: p. 258-303.

157. Alvarenga, S.A., et al., *A general survey and some taxonomic implications of diterpenes in the Asteraceae.* Botanical Journal of the Linnean Society, 2005. **147**(3): p. 291-308.

158. Gottlieb, O.R., *Evolution of Natural Products*, in *Natural Products of Woody Plants: Chemicals Extraneous to the Lignocellulosic Cell Wall*, J.W. Rowe, Editor. 1989, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 125-153.

159. Sparg, S.G., M.E. Light, and J. van Staden, *Biological activities and distribution of plant saponins.* Journal of Ethnopharmacology, 2004. **94**(2): p. 219-243.

160. Faizal, A. and D. Geelen, *Saponins and their role in biological processes in plants.* Phytochemistry Reviews, 2013. **12**(4): p. 877-893.

161. Ur Rashid, M., et al., *The chemistry and pharmacology of alkaloids and allied nitrogen compounds from Artemisia species: A review.* Phytother Res, 2019. **33**(10): p. 2661-2684.

162. Waller, G.R. and E.K. Nowacki, *The role of alkaloids in plants*, in *Alkaloid Biology and Metabolism in Plants*. 1978, Springer. p. 143-181.

163. Waller, G., *Alkaloid biology and metabolism in plants*. 2012: Springer Science & Business Media.

164. Kurek, J., *Introductory Chapter: Alkaloids-Their Importance in Nature and for Human Life*, in *Alkaloids-Their Importance in Nature and Human Life*. 2019, IntechOpen.

165. Gomberg, M., *ON THE ACTION OF WAGNER'S REAGENT UPON CAFFEINE AND A NEW METHOD FOR THE ESTIMATION OF CAFFEINE.* Journal of the American Chemical Society, 1896. **18**(4): p. 331-342.

166. Isah, T., *Stress and defense responses in plant secondary metabolites production.* Biological Research, 2019. **52**(1): p. 39.

167. Fu, Z.-X., et al., *A comprehensive generic-level phylogeny of the sunflower family:*

Implications for the systematics of Chinese Asteraceae. China Phylogeny Consortium, 2016. **54**(4): p. 416-437.

168. Funk, V.A., et al., *Classification of compositae.* Systematics, evolution, biogeography of Compositae, 2009.

169. Funk, V., et al., *Systematics, Evolution, and Biogeography of Compositae*. 2009, Veinna, Austria: International Association for Plant Taxonomy, Institue of Botany, University of Vienna.

170. Zdero, C. and F. Bohlmann, *Systematics and evolution within theCompositae, seen with the eyes of a chemist.* Plant Systematics and Evolution, 1990. **171**(1): p. 1-14.

171. B, P.P. and W. L, *Systematics, evolution, biogeography of Compositae: Introduction to Asteroideae* 2009. **495**: p. 502.

172. Alvarenga, S.A.V., et al., *A general survey and some taxonomic implications of diterpenes in the Asteraceae.* Botanical Journal of the Linnean Society, 2005. **147**(3): p. 291-308.

173. Chen, T., et al., *The complete chloroplast genome of Crossostephium chinense (Asteraceae), using genome skimming data.* Mitochondrial DNA Part B, 2019. **4**(1): p. 322-323.

174. Kowiyou, Y., D.B. H, and M.A. Muthama, *Phylogenetic exploration of commonly used medicinal plants in S outh Africa.* Mol Ecol Resour, 2015. **15**(2): p. 405-413.

175. Mellergaard, L.M., et al., *Using a phylogenetic approach to selection of target plants in drug discovery of acetylcholinesterase inhibiting alkaloids in Amaryllidaceae tribe Galantheae.* Biochemical Systematics and Ecology, 2010. **38**(5): p. 1026-1034.

176. Numonov, S., et al., *Assessment of artemisinin contents in selected Artemisia species from Tajikistan (central Asia).* Medicines, 2019. **6**(1): p. 23.

APPENDIX I

CURRENT CLASSIFICATION OF ASTERACEAE [168]

No	Sub-family	Tribe
1	**Barnadesioideae** (D. Don) Bremer & Jansen 1992	Barnadesieae D. Don 1830
2	**Stifftioideae** (D. Don) Panero 2007	Stifftieae D. Don 1830
3	**Mutisioideae** (Cass.) Lindl 1829	Mutisieae Cass 1819
		Onoserideae Bentham, Panero & Funk 2007
4	**Wunderlichioideae** Panero & Funk 2007	Wunderlichieae Panero & Funk 2007
		Hyalideae Panero 2007
5	**Gochnatioideae** Benth & Hook; Panero & Funk 2002	Gochnatieae Benth & Hook; Panero & Funk 2002
6	**Hecastocleidoideae** Panero & Funk 2002	Hecastocleideae Panero & Funk 2002
7	**Carduiodae** Cass Sweet 1826	Dicomeae Panero & Funk 2002
		Oldenburgieae Ortiz 2009
		Tarchonantheae Kostel 1883
		Cardueae Cass 1819
8	**Pertyoideae** Panero & Funk 2002	Pertyeae Panero & Funk 2002
9	**Gymnarrhenoideae** Panero & Funk 2002	Gymnarrheneae Panero & Funk 2002
10	**Cichoriodeae** Juss Chevall 1828	Cichorieae Lam & DC 1860
		Arctotideae Cass 1819
		Eremonthamneae H.Rob & Brettell 1973
		Liabeae Cass x Dumort 1927
		Vernonieae Cass 1918
		Platycarpheae Funk & H Rob 2009
		Moquinieae H Rob 1994
11	**Corymbioideae** Panero & Funk 2002	Corymbieae Panero & Funk 2002

12	**Asteroideae** Cass Lindl 1829	Senecioneae Cass 1819
		Calenduleae Cass 1819
		Gnaphalieae Cass Lecoq & Juillet 1831
		Astereae Cass 1819
		Anthemideae Cass 1819
		Inuleae Cass 1819
		Athroismeae Panero 2002
	Heliantheae Alliance	Feddeeae Pruski 2008
		Helenieae Lindl 1829
		Coreopsideae Lindl 1829
		Neurolaeneae Rydb 1927
		Tagetae Cass 1819
		Chaenactideae BG Baldwin 2002
		Bahieae BG Baldwin 2002
		Polymnieae Panero 2002
		Heliantheae Cass 1819
		Millerieae Lindl 1929
		Perityleae BG Baldwin 2002
		Eupatorieae Cass 1819

APPENDIX II

Ethnomedicinal Plants Working List for Phylogenetic Mapping

No	Acession NCBI	Family	Genus	Species	Indigenous culture	note	Disease
1	0	Acanthaceae	*Hygrophila*	*Hygrophila auriculata*	West bengal	West bengal	Diarrhea
2	0	Acanthaceae	*Adhatoda*	*Adhatoda zeylanica*	India	India	Fever
3	GQ465765.1	Acanthaceae	*Asystasia*	*Asystasia gangetica*	Uganda	Uganda	Fever
4	EU528907.1	Acanthaceae	*Phlogacanthus*	*Phlogacanthus thyrsiflorus*	Northeast India	Northeast India	Fever
5	0	Acanthaceae	*Justicia*	*Justicia anselliana (Nees)* T. Anderson	Uganda	Uganda	Malaria
6	KC991048.1	Acanthaceae	*Justicia*	*Justicia spp.*	Uganda	Uganda	Malaria
7	0	Acanthaceae	*Monechma*	*Monechma subsessile* C. B. Clarke	Uganda	Uganda	Malaria
8	0	Acanthaceae	*Strobilanthes*	*Strobilanthes auriculatus* Nees	Northeast india	Northeast india	Malaria
9	KJ718259.1	Acanthaceae	*Thunbergia*	*Thunbergia alata Sims/Alternaria thunbergiae*	Uganda	Uganda	Malaria
10	KC441008.1	Acanthaceae	*Andrographis*	*Andrographis paniculata* Wall. Ex Nees	Multicultural	Northeast india, Bangladesh, Nepal	Malaria, Fever
11	KM034015.1	Acanthaceae	*Blepharis*	*Blepharis diversispina* (Nees) C.B.Clarke SSS99	Limpopo	Limpopo	Tuberculosis
12	0	Adiantiaceae	*Adiantum*	*Adiantum capillaris-veneris* L	Multicultural	Nepal, Limpopo	Fever, Tuberculosis
13	0	Agapanthaceae	*Agapanthus*	*Agapanthus inapertus* P. Beav	Limpopo	Limpopo	Tuberculosis
14	KU692183.1	Alliaceae	*Tulbaghia*	*Tulbaghia violacea* Harv. var.	Limpopo	Limpopo	Tuberculosis
15	0	Aloaceae	*Aloe*	*Aloe dawei* A. Berger	Uganda	Uganda	Malaria
16	0	Aloaceae	*Aloe*	*Aloe ferox* Mill	Uganda	Uganda	Malaria
17	0	Aloaceae	*Aloe*	*Aloe kedongensis*	Uganda	Uganda	Malaria
18	0	Aloaceae	*Aloe*	*Aloe latertia*	Uganda	Uganda	Malaria
19	0	Aloaceae	*Aloe*	*Aloe volkensii*	Uganda	Uganda	Malaria
20	KY968942.1	Amaranthaceae	*Celosia*	*Celosia cristata*	India	India	Diarrhea
21	0	Amaranthaceae	*Alternanthera*	*Alternanthera sessiles DC/Alternanthera halimifolia*	Nepal	Nepal	Fever
22	0	Amaranthaceae	*Amaranthus*	*Amaranthus spinosus* L	Bangladesh	Bangladesh	Fever
23	0	Amaranthaceae	*Deeringia*	*Deeringia amaranthoides*	Meghalaya	Meghalaya	Fever
24	MH768066.1	Amaranthaceae	*Achyranthes*	*Achyranthes aspera*	Multicultural	India, bangladesh	Fever, Diarrhea
25	KY968931.1	Amaranthaceae	*Amaranthus*	*Amaranthus spp.*	Multicultural	Uganda, Kenya, Bangladesh	Malaria
26	JQ403571.1	Amaranthaceae	*Alternanthera*	*Alternanthera spp.*	Multicultural	Ivory coast, Nepal, West bengal	Malaria, Diarrhea
27	KJ833767.1	Amaranthaceae	*Mangifera*	*Mangifera indica* L.	Multicultural	Uganda, Ghana, Nigeria, Ivory coast, Maputaland, Nepal	Malaria, Fever, Tuberculosis, Diarrhea
28	KC747456.1	Amaranthaceae	*Celosia*	*Celosia trigyna* L	Uganda	Uganda	Tuberculosis

29	0	Amaryllidaceae	*Brunsvigia*	*Brunsvigia grandiflora*	Bizana	Bizana	Diarrhea
30	AY139137.1	Amaryllidaceae	*Crinum*	*Crinum latifolium*	West bengal	West bengal	Diarrhea
31	FJ664287.1	Amaryllidaceae	*Allium spp.*	*Allium spp.*	Multicultural	Uganda, Nigeria	Malaria, Fever
32	0	Anacardiaceae	*Lannea*	*Lannea edulis*	Zimbabwe	Zimbabwe	Diarrhea
33	0	Anacardiaceae	*Ozoroa*	*Ozoroa insignis*	Zimbabwe	Zimbabwe	Diarrhea
34	MN257741.1	Anacardiaceae	*Ozoroa*	*Ozoroa sphaerocarpa*	Limpopo	Limpopo	Diarrhea
35	0	Anacardiaceae	*Searsia*	*Searsia chirindensis*	Bizana	Bizana	Diarrhea
36	0	Anacardiaceae	*Semicarpus*	*Semicarpus anacardium*	India	India	Diarrhea
37	0	Anacardiaceae	*Lannea*	*Lannea schweinfurthii* (Engl.) Engl	Kenya	Kenya	Malaria
38	0	Anacardiaceae	*Rhus*	*Rhus natalensis* Bernh. Ex Krauss	multicultural	Uganda, Kenya	Malaria
39	KF664192.1	Anacardiaceae	*Anacardium*	*Anacardium occidentale* L.	Multicultural	Nigeria, South-western Nigeria, Senegal	Malaria, Fever, Tuberculosis
40	0	Anacardiaceae	*Rhus*	*Rhus vulgaris* Meikle	Uganda	Uganda	Malaria, Tuberculosis
41	0	Anacardiaceae	*Pseudospondia*	*Pseudospondia microcarpa* (A. Rich.) Engl.	Uganda	Uganda	Tuberculosis
42	AY641512.1	Anacardiaceae	*Schinus*	*Schinus molle* L	Limpopo	Limpopo	Tuberculosis
43	0	Anacardiaceae	*Sclerocarya*	*Sclerocarya birrea* (A.Rich.) Hochst. subsp. caffra	Multicultural	Limpopo	Tuberculosis, Diarrhea
44	0	Annonaceae	*Pachypodanthium*	*Pachypodanthium staudlii*	Ghana	Ghana	Fever
45	0	Annonaceae	*Cleistopholis*	*Cleistopholis patens* Engl. &Diels. (GOM 23	Ghana	Ghana	Malaria
46	0	Annonaceae	*Greenwayodendron*	*Greenwayodendron sp.*	Ghana	Ghana	Malaria
47	MN114134.1	Annonaceae	*Uvaria*	*Uvaria acuminata* Oliv.	Kenya	Kenya	Malaria
48	0	Annonaceae	*Uvaria*	*Uvaria afzelii* G.F. Scott-Ellio	Ivory coast	Ivory coast	Malaria
49	0	Annonaceae	*Uvaria*	*Uvaria schefflera* Diels	Kenya	Kenya	Malaria
50	0	Annonaceae	*Annona*	*Annona muricata* L	Indonesia	Indonesia	Malaria, Fever
51	0	Annonaceae	*Enantia*	*Enantia chlorantha* Oliv	Nigeria	Nigeria	Malaria, Fever
52	0	Annonaceae	*Xylopia*	*Xylopia aethiopica*	Nigeria	Nigeria	Malaria, Fever
53	AF337187.1	Apiaceae	*Lagoecia*	*Lagoecia cuminoides*	Iran	Iran	Diarrhea
54	KY411878.1	Apiaceae	*Trachyspermum*	*Trachyspermum ammi*	Nepal	Nepal	Diarrhea
55	0	Apiaceae	*Heracleum*	*Heracleum pinnatum* C.B. Clarke	Nepal	Nepal	Fever
56	EU185678.1	Apiaceae	*Heracleum*	*Heracleum spp.*	Nepal	Nepal	Fever
57	0	Apiaceae	*Alstonia*	*Alstonia boonei* De Wild	Multicultural	Uganda, Ghana	Malaria
58	0	Apiaceae	*Heteromorpha*	*Heteromorpha trifoliata* Eckl. & Zeyh.	Uganda	Uganda	Malaria

59	KR215628.1	Apiaceae	*Centella*	*Centella asiatica* (L.) Urb	Multicultural	Uganda, Nepal, Kenya, Bangladesh	Malaria, Fever, Tuberculosis, Diarrhea
60	AM158945.1	Apiaceae	*Alepidea*	*Alepidea amatymbica* Eckl. & Zeyh. v	Limpopo	Limpopo	Tuberculosis
61	U27578.2	Apiaceae	*Heteromopha*	*Heteromopha arborescens var frutescens*	Limpopo	Limpopo	Tuberculosis
62	AM748814.1	Apiaceae	*Steganotaenia*	*Steganotaenia araliacea* Hochst	Uganda	Uganda	Tuberculosis
63	0	Apocynaceae	*Carissa*	*Carissa bispinosa*	Zimbabwe	Zimbabwe	Diarrhea
64	AJ492817.1	Apocynaceae	*Sarcostemma*	*Sarcostemma viminale/Cynanchum viminale*	Maputaland	Maputaland	Diarrhea
65	0	Apocynaceae	*Hedranthera*	*Hedranthera barteri* Hook. f.	Nigeria	Nigeria	Fever
66	DQ916852.1	apocynaceae	*Mondia*	*Mondia whitei*	Ghana	Ghana	Fever
67	MH566887.1	Apocynaceae	*Allamanda*	*Allamanda cathartica* L.	Nigeria	Nigeria	Malaria
68	0	Apocynaceae	*Alstonia*	*Alstonia congensis* Engl	Nigeria	Nigeria	Malaria
69	0	Apocynaceae	*Carissa*	*Carissa spinarum* Lodd. ex A. DC	Uganda	Uganda	Malaria
70	0	Apocynaceae	*Diplorhynchus*	*Diplorhynchus condylocarpon* (Müll.Arg.) Pichon	Zimbabwe	Zimbabwe	Malaria
71	KC189049.1	Apocynaceae	*Funtumia*	*Funtumia africana* (Benth.)	Nigeria	Nigeria	Malaria
72	0	Apocynaceae	*Funtumia*	*Funtumia elastica* (Preuss) Stapf	Ivory coast	Ivory coast	Malaria
73	0	Apocynaceae	*Landolphia*	*Landolphia sp.*	Ghana	Ghana	Malaria
74	0	Apocynaceae	*Laudolphia*	*Laudolphia buchananii* (Hall.f) Stapf.	Kenya	Kenya	Malaria
75	0	Apocynaceae	*Melodinus*	*Melodinus monogynus* Roxb	Northeast india	Northeast india	Malaria
76	0	Apocynaceae	*Rauvolfia*	*Rauvolfia mombasiana* Stapf	Kenya	Kenya	Malaria
77	JX856518.1	Apocynaceae	*Tabernaemontana*	*Tabernaemontana elegans* Stapf	Zimbabwe	Zimbabwe	Malaria
78	MN177158.1	Apocynaceae	*Carissa*	*Carissa spp.*	Multicultural	Uganda , Kenya, Zimbabwe, Limpopo	Malaria, Diarrhea
79	MN257723.1	Apocynaceae	*Holarrhena*	*Holarrhena pubescens* Wall. Ex G. Don	Multicultural	Zimbabwe, India	Malaria, Diarrhea
80	0	Apocynaceae	*Alstonia*	*Alstonia boonei* De Wild.	Multicultural	Nigeria, Ivory coast	Malaria, Fever
81	0	Apocynaceae	*Picralima*	*Picralima nitida* Th. & H.Dur	Ghana	Ghana	Malaria, Fever
82	KC878601.1	Apocynaceae	*Rauvolfia*	*Rauvolfia vomitoria* Afz	Multicultural	Nigeria, Ivory coast	Malaria, Fever
83	MH566880.1	Apocynaceae	*Alstonia*	*Alstonia scholaris* R.Br	Multicultural	Northeast india, Indonesia, Bangladesh, Nepal, West bengal	Malaria, Fever, Diarrhea
84	HQ130657.2	Apocynaceae	*Catharanthus*	*Catharanthus roseus* G. Don	Multicultural	Uganda, Zimbabwe, Maputaland	Malaria, Tuberculosis, Diarrhea

85	0	Apocynaceae	*Strophanthus*	*Strophanthus speciosus* (Ward & Harv.) Reber	Limpopo	Limpopo	Tuberculosis
86	0	Araceae	*Culcasia*	*Culcasia faleifolia* Eng	Uganda	Uganda	Malaria
87	0	Araceae	*Homalomena*	*Homalomena rubra* Hassk	Indonesia	Indonesia	Malaria
88	0	Araceae	*Pothos*	*Pothos ovatifolius* Engl	Indonesia	Indonesia	Malaria
89	FJ874937.1	Araceae	*Acorus*	*Acorus calamus* L.	Multicultural	Northeast india, Nepal, Bangladesh	Malaria, Fever, Diarrhea
90	0	Araceae	*Stylochaeton*	*Stylochaeton natalensis* Schott	Limpopo	Limpopo	Tuberculosis
91	0	Araceae	*Zantedeshia*	*Zantedeshia aethiopica* (L.)	Limpopo	Limpopo	Tuberculosis
92	U63186.1	Araliaceae	*Hedera*	*Hedera helix* L	Iran	Iran	Fever
93	MK978665.1	Araliaceae	*Schefflera*	*Schefflera sp.*	Indonesia	Indonesia	Malaria, Fever
94	HQ265520.1	Arecaceae	*Elaeis*	*Elaeis guineensis* Jacq	Nigeria	Nigeria	Fever
95	MK683071.1	Arecaceae	*Phoenix*	*Phoenix reclinata* Jacq	Nigeria	Nigeria	Fever
96	HQ265515.1	Arecaceae	*Cocos*	*Cocos nucifera* L.	Multicultural	Ghana, nigeria	Malaria, Fever
97	0	Aristolochiaceae	*Aristolochia*	*Aristolochia heppii* Merxm	Zimbabwe	Zimbabwe	Malaria
98	0	Aristolochiaceae	*Aristolochia*	*Aristolochia spp.*	Zimbabwe	Zimbabwe	Malaria
99	0	Aristolochiaceae	*Aristolochia*	*Aristolochia tomentosa* Sims.	Uganda	Uganda	Malaria
100	KM092118.1	Aristolochiaceae	*Aristolochia*	*Aristolochia spp.*	Multicultural	Uganda, Zimbabwe	Malaria, Tuberculosis
101	DQ916851.1	Asclepiadaceae	*Hemidesmus*	*Hemidesmus indicus*	India	India	Diarrhea
102	AM396851.1	Asclepiadaceae	*Pergularia*	*Pergularia daemia*	India	India	Fever, Diarrhea
103	GQ465764.1	Asclepiadaceae	*Gomphocarpus*	*Gomphocarpus physocarpus* E. Mey.	Uganda	Uganda	Malaria
104	KP764850.1	Asclepiadaceae	*Parquetina*	*Parquetina nigrescens* (Afzel.) Bullock	Ivory coast	Ivory coast	Malaria
105	0	Asparagaceae	*Dracaena*	*Dracaena steudneri* Engl	Uganda	uganda	Tuberculosis
106	AM396851.1	Aspecidaceae	*Daemia*	*Daemia extensa/Pergularia daemia*	India	India	Fever
107	KP072742.1	Asphodelaceae	*Aloe*	*Aloe spp.*	Multicultural	Uganda, Kenya, Nigeria, Bizana	Malaria, Fever, Tuberculosis, Diarrhea
108	0	Asphodelaceae	*Aloe*	*Aloe falcata Baker*	Limpopo	Limpopo	Tuberculosis
109	0	Aspleniaceae	*Asplenium*	*Asplenium adiantoides* C. Chr	Northeast india	Northeast india	Malaria
110	KY968839.1	Asteraceae	*Ageratina*	*Ageratina adenophora*	Meghalaya	Meghalaya	Diarrhea
111	0	Asteraceae	*Artemisia*	*Artemisia parviflora*	West bengal	West bengal	Diarrhea
112	EU527198.1	Asteraceae	*Berkheya*	*Berkheya bipinnatifida*	Bizana	Bizana	Diarrhea

113	KF443296.1	Asteraceae	*Blumea*	*Blumea spp*	West bengal	West bengal	Diarrhea
114	KY909250.1	Asteraceae	*Mikania*	*Mikania micrantha*	Meghalaya	Meghalaya	Diarrhea
115	0	Asteraceae	*Launaea*	*Launaea pinnatifida*	India	India	Diarrhea
116	0	Asteraceae	*Vernonia*	*Vernonia amygdalina*	Congo	Congo	Diarrhea
117	AY603185.1	Asteraceae	*Achillea*	*Achillea millefolium* Linn	Nepal	Nepal	Fever
118	0	Asteraceae	*Artemisia*	*Artemisia brevifolia* Wall.	Nepal	Nepal	Fever
119	0	Asteraceae	*Artemisia*	*Artemisia gmelinii* Web. ex Stechm	Nepal	Nepal	Fever
120	0	Asteraceae	*Aster*	*Aster diplostephioides* Benth	Nepal	Nepal	Fever
121	0	Asteraceae	*Aster*	*Aster tibeticus* Hk. f	Nepal	Nepal	Fever
122	MH711524.1	Asteraceae	*Atractylodes*	*Atractylodes lancea*	Thailand	Thailand	Fever
123	KY397481.1	Asteraceae	*Carthamus*	*Carthamus tinctorius* L.	Iran	Iran	Fever
124	KY676855.1	Asteraceae	*Centaurea*	*Centaurea depressa* M Bieb	Nepal	Nepal	Fever
125	0	Asteraceae	*Chrysanthemum*	*Chrysanthemum pyrethroides* (Kar. & Kir.) B. Fedtsch	Nepal	Nepal	Fever
126	JQ230974.1	Asteraceae	*Cichorium*	*Cichorium intybus* L	Iran	Iran	Fever
127	AY723272.1	Asteraceae	*Cremanthodium*	*Cremanthodium ellisii* (Hk. f.) Kitam	Nepal	Nepal	Fever
128	0	Asteraceae	*Eclipta*	*Eclipta erecta*	India	India	Fever
129	KF454311.1	Asteraceae	*Inula*	*Inula racemosa* Hk. f	Nepal	Nepal	Fever
130	0	Asteraceae	*Inula*	*Inula rhizocephala Shrenk. var. rhizocephaloides* (Cl.) Kitam.	Nepal	Nepal	Fever
131	AY914821.1	Asteraceae	*Saussurea*	*Saussurea lappa* C.B. Clarke/*Saussurea costus*	Nepal	Nepal	Fever
132	0	Asteraceae	*Tanacetum*	*Tanacetum dolichophyllum* (Kitam.) Kitam	Nepal	Nepal	Fever
133	0	Asteraceae	*Tanacetum*	*Tanacetum gracile* Hk. f. & T	Nepal	Nepal	Fever
134	0	Asteraceae	*Waldheimia*	*Waldheimia stoliczkai* (C.B. Clarke.) *Osterf*	Nepal	Nepal	Fever
135	0	Asteraceae	*Eclipta*	*Eclipta prostrata*	Nepal	Nepal	Fever, Diarrhea
136	FJ696965.1	Asteraceae	*Acanthospermum*	*Acanthospermum hispidum* (DC) Kuntze	Multicultural	Ivory coast, Ghana	Malaria
137	0	Asteraceae	*Artemisia*	*Artemisia nilagirica* (C.B. Clarke) Pamp	Northeast india	Northeast india	Malaria
138	0	Asteraceae	*Aspilia*	*Aspilia africana* (Pers.) C. D. Adams	Uganda	Uganda	Malaria
139	EF155745.1	Asteraceae	*Baccharoides*	*Baccharoides adoensis* (Sch. Bip. ex Walp.) H. Rob.	Multicultural	Uganda , Zimbabwe	Malaria
140	0	Asteraceae	*Bidens*	*Bidens grantii* Sherf	Uganda	Uganda	Malaria

141	0	Asteraceae	*Bothriocline*	*Bothriocline longipes* N. E. Br.	Uganda	Uganda	Malaria
142	0	Asteraceae	*Brachylaena*	*Brachylaena huillensis* O. Hoffm	Zimbabwe	Zimbabwe	Malaria
143	0	Asteraceae	*Chrysanthemum*	*Chrysanthemum sp.*	Indonesia	Indonesia	Malaria
144	0	Asteraceae	*Conyza*	*Conyza floribunda* H. B. K.	Uganda	Uganda	Malaria
145	AF118513.1	Asteraceae	*Conyza*	*Conyza spp.*	Uganda	Uganda	Malaria
146	0	Asteraceae	*Conyza*	*Conyza sumatrensis* (Retz.) E. H. Walker	Uganda	Uganda	Malaria
147	MN723919.1	Asteraceae	*Crassocephalum*	*Crassocephalum vitellinum*	Uganda	Uganda	Malaria
148	MF349157.1	Asteraceae	*Emilia*	*Emilia javanica* (Burm. F.) C. B. Rob.	Uganda	Uganda	Malaria
149	0	Asteraceae	*Erigeron*	*Erigeron floribundus* (Kunth)	Ivory coast	Ivory coast	Malaria
150	0	Asteraceae	*Erythrocephalum*	*Erythrocephalum zambesianum* Oliv. & Hiern	Zimbabwe	Zimbabwe	Malaria
151	0	Asteraceae	*Guizotia*	*Guizotia scabra* Chiov.	Uganda	Uganda	Malaria
152	0	Asteraceae	*Gynura*	*Gynura scandens* O. Hoffm	Uganda	Uganda	Malaria
153	0	Asteraceae	*Helianthus*	*Helianthus annus* L	Northeast india	Northeast india	Malaria
154	0	Asteraceae	*Melanthera*	*Melanthera scandens* (Schumach. & ,onn.) Roberty	Multicultural	Uganda, ivory coast	Malaria
155	0	Asteraceae	*Microglossa*	*Microglossa pyrifolia* (Lam.)O. Ktze	Multicultural	Uganda , Ivory coast	Malaria
156	MK261253.1	Asteraceae	*Pluchea*	*Pluchea ovalis* DC.	Uganda	Uganda	Malaria
157	KP972321.1	Asteraceae	*Schkuhria*	*Schkuhria pinnata* (Lam.)	Uganda	Uganda	Malaria
158	0	Asteraceae	*Senecio*	*Senecio syringitolius* O.Hoffman.	Kenya	Kenya	Malaria
159	JN987228.1	Asteraceae	*Sigesbeckia*	*Sigesbeckia orientalis* L.	Uganda	Uganda	Malaria
160	AF459923.1	Asteraceae	*Solanecio*	*Solanecio mannii* (Hook. f.) C. Jeffrey	Uganda	Uganda	Malaria
161	AY458002.1	Asteraceae	*Sonchus*	*Sonchus oleraceus* L.	Uganda	Uganda	Malaria
162	KC800429.1	Asteraceae	*Tagetes*	*Tagetes minuta* L	Uganda	Uganda	Malaria
163	0	Asteraceae	*Vernonia*	*Vernonia lasiopus* O. Hoffm/*Baccharoides lasiopus*	Uganda	Uganda	Malaria
164	KY215735.1	Asteraceae	*Xanthium*	*Xanthium strumarium* L.	Northeast india	Northeast india	Malaria
165	MH768114.1	Asteraceae	*Tridax*	*Tridax procumbens* L.	Multicultural	Kenya, India	Malaria, Diarrhea
166	0	Asteraceae	*Blumea*	*Blumea pubigera* (L.) Merr	Indonesia	Indonesia	Malaria, Fever
167	MH768097.1	Asteraceae	*Chromolaena*	*Chromolaena odorata* L	Nigeria	Nigeria	Malaria, Fever
168	KM887383.1	Asteraceae	*Taraxacum*	*Taraxacum officinale* Wigg.	multicultural	Northeast india, nepal	Malaria, Fever
169	KT965671.1	Asteraceae	*Artemisia*	*Artemisia spp.*	Multicultural	Uganda, Thailand, Nigeria, West bengal	Malaria, Fever, Diarrhea
170	MH398898.1	Asteraceae	*Aster*	*Aster spp.*	Multicultural	Northeast india, Nepal, Bizana	Malaria, Fever, Diarrhea

171	KR425612.1	Asteraceae	*Ageratum*	*Ageratum conyzoides* L.	Multicultural	Uganda , Nigeria, Nepal	Malaria, Fever, Tuberculosis
172	KY968897.1	Asteraceae	*Bidens*	*Bidens pilosa* L	Multicultural	Uganda , nepal	Malaria, Fever, Tuberculosis
173	AY504695.1	Asteraceae	*Vernonia*	*Vernonia spp./Gymnanthemum spp.*	Multicultural	Uganda, Nigeria, Ghana, Congo	Malaria, Fever, Tuberculosis, Diarrhea
174	0	Asteraceae	*Artemisia*	*Artemisia afra* Jacq. ex Willd	Multicultural	Uganda, Limpopo	Malaria, Tuberculosis
175	MH050186.1	Asteraceae	*Tithonia*	*Tithonia diversifolia* A. Gray	Multicultural	Uganda, Indonesia	Malaria, Tuberculosis
176	0	Asteraceae	*Vernonia*	*Vernonia cinerea* (L.) Less.	Uganda	Uganda	Malaria, Tuberculosis
177	0	Asteraceae	*Aspilia*	*Aspilia africana* (Pers.) C.D. Adams	Uganda	Uganda	Tuberculosis
178	0	Asteraceae	*Brachylaena*	*Brachylaena transvaalensis*	Limpopo	Limpopo	Tuberculosis
179	KT865463.1	Asteraceae	*Callilepis*	*Callilepis laureola* DC	Limpopo	Limpopo	Tuberculosis
180	JX524599.1	Asteraceae	*Gnaphalium*	*Gnaphalium purpureum* L/*Gamochaeta purpurea*	Uganda	Uganda	Tuberculosis
181	AF046954.1	Asteraceae	*Psiadia*	*Psiadia punctulata* (DC.) Vatke	Limpopo	Limpopo	Tuberculosis
182	0	Asteraceae	*Senecio*	*Senecio serratuloides* DC	Limpopo	Limpopo	Tuberculosis
183	0	Asteraceae	*Dicoma*	*Dicoma anomala* subsp. Gerrardii	Limpopo	Limpopo	Tuberculosis, Diarrhea
184	0	Asteraceae	*Helichrysum*	*Helichrysum spp*	Limpopo	Limpopo	Tuberculosis, Diarrhea
185	0	Asteraceae	*Vernonia*	*Vernonia natalensis*	Limpopo	Limpopo	Tuberculosis, Diarrhea
186	EU436863.1	Averrhoaceae	*Averrhoa*	*Averrhoa carambola* L	Bangladesh	Bangladesh	Fever
187	0	Balsaminaceae	*Impatiens*	*Impatiens angustifolia* Blume	Northeast india	Northeast india	Malaria
188	0	Begoniaceae	*Begonia*	*Begonia rubrovenia*	Meghalaya	Meghalaya	Diarrhea
189	AF328965.1	Berberidaceae	*Podophyllum*	*Podophyllum hexandrum* Royle	Nepal	Nepal	Fever
190	HM347891.1	Berberidaceae	*Berberis*	*Berberis aristata* DC.	Multicultural	Northeast india, Himalaya	Malaria, Diarrhea
191	0	Berberidaceae	*Berberis*	*Berberis lyceum* Royle	Nepal	Nepal	Malaria, Fever
192	AJ783641.1	Betulaceae	*Betula*	*Betula alnoides* Buch.-Ham	Northeast india	Northeast india	Malaria
193	FJ606747.1	Bignoniaceae	*Oroxylum*	*Oroxylum indicum*	Multicultural	Meghalaya, nepal	Diarrhea
194	0	Bignoniaceae	*Stereospermum*	*Stereospermum kunthianu*	Nigeria	Nigeria	Diarrhea
195	AY695862.1	Bignoniaceae	*Tecomaria*	*Tecomaria capensis*	India	India	Diarrhea
196	AY178636.1	Bignoniaceae	*Tecoma*	*Tecoma spp*	Multicultural	India, Bizana	Fever, Diarrhea
197	JN115030.1	Bignoniaceae	*Kigelia*	*Kigelia africana* (Lam.) Benth.	Multicultural	Nigeria, Uganda	Fever, Tuberculosis
198	0	Bignoniaceae	*Markhamia*	*Markhamia lutea* (Benth.) K. Schum	Uganda	Uganda	Malaria

199	MF616581.1	Bignoniaceae	*Spathodea*	*Spathodea campanulata* Buch. -Harm. ex DC	Multicultural	Uganda, Ghana, Limpopo	Malaria, Tuberculosis
200	KF055235.1	Bixaceae	*Bixa*	*Bixa orellana* L	Nigeria	Nigeria	Malaria
201	0	Bombaceae	*Bombax*	*Bombax ceiba*	Nepal	Nepal	Diarrhea
202	0	Bombaceae	*Adansonia*	*Adansonia digitate* Linn.	Kenya	Kenya	Malaria
203	KM453169.1	Bombaceae	*Ceiba*	*Ceiba pentandra* (L.) Gaertn	Nigeria	Nigeria	Malaria
204	HQ658376.1	Bombaceae	*Bombax*	*Bombax spp*	Multicultural	Ghana, Nepal	Malaria, Diarrhea
205	0	Bombaceae	*Eriodendron*	*Eriodendron anfractosum* D.C.	Indonesia	Indonesia	Malaria, Fever
206	0	Boraginaceae	*Cordia*	*Cordia fragantissima*	Meghalaya	Meghalaya	Diarrhea
207	0	Boraginaceae	*Arnebia*	*Arnebia guttata Bunge*	Nepal	Nepal	Fever
208	EF199867.2	Boraginaceae	*Arnebia*	*Arnebia spp.*	Nepal	Nepal	Fever
209	MH768074.1	Boraginaceae	*Heliotropium*	*Heliotropium indicum* L	Bangladesh	Bangladesh	Fever
210	JF332094.1	Boraginaceae	*Cordia*	*Cordia spp*	Multicultural	Iran, Meghalaya	Fever, Diarrhea
211	0	Boraginaceae	*Cynoglossum*	*Cynoglossum glochidion Wall*	Northeast india	Northeast india	Malaria
212	GQ268080.1	Brassicaceae	*Capsella*	*Capsella bursa-pastoris* L	Nepal	Nepal	Fever
213	AY254531.1	Brassicaceae	*Nasturtium*	*Nasturtium officinale* Br	Northeast india	Northeast india	Malaria
214	0	Bromeliaceae	*Ananas*	*Ananas comosus* Linn	Multicultural	Nigeria, Ghana, Bangladesh	Malaria, Fever
215	JN882677.1	Burseraceae	*Commiphora*	*Commiphora marlothii*	Limpopo	Limpopo	Diarrhea
216	0	Burseraceae	*Dacryodes*	*Dacryodes edulis* (G. Don.) H.J. Lam	Nigeria	Nigeria	Malaria
217	0	Burseraceae	*Canarium*	*Canarium schweinfurthii* Engl	Uganda	Uganda	Tuberculosis
218	0	Caesalpiniaceae	*Bauhinia*	*Bauhinia purpurea*	Bangladesh	Bangladesh	Diarrhea
219	JX856406.1	Caesalpiniaceae	*Bauhinia*	*Bauhinia spp*	Multicultural	himalaya, meghalaya	Diarrhea
220	0	Caesalpiniaceae	*Caesalpinia*	*Caesalpinia sepiaria*	India	India	Diarrhea
221	0	Caesalpiniaceae	*Senna*	*Senna sceptabilis*	Nigeria	Nigeria	Fever
222	MG949357.1	Caesalpiniaceae	*Tamarindus*	*Tamarindus indica*	India	India	Fever
223	MG949374.1	Caesalpiniaceae	*Anthonotha*	*Anthonotha macrophylla* P. Beauv	Ivory coast	Ivory coast	Malaria
224	0	Caesalpiniaceae	*Cassia*	*Cassia alata Linn/Senna alata*	Multicultural	Ivory coast, Ghana	Malaria
225	0	Caesalpiniaceae	*Cassia*	*Cassia hirsuta/Senna hirsuta*	Uganda	Uganda	Malaria
226	KT279731.1	Caesalpiniaceae	*Chamaecrista*	*Chamaecrista nigricans* Greene	Uganda	Uganda	Malaria
227	0	Caesalpiniaceae	*Erythrophleum*	*Erythrophleum pyrifolia*	Uganda	Uganda	Malaria
228	0	Caesalpiniaceae	*Senna*	*Senna siamea* (Lam.)	Multicultural	nigeria, Ivory coast	Malaria
229	0	Caesalpiniaceae	*Senna*	*Senna spectabilis* (DC.) H. S. Irwin & Barneby	Uganda	Uganda	Malaria

230	MF963893.1	Caesalpiniaceae	*Senna*	*Senna spp.*	Multicultural	Uganda, Ivory coast, Ghana, Kenya, Nigeria, Iran, Zimbabwe, Limpopo, Maputaland, India	Malaria, Fever, Tuberculosis, Diarrhea
231	0	Caesalpiniaceae	*Cassia*	*Cassia occidentalis* Linn/*Senna occidentalis*	Multicultural	Ivory coast, Kenya, Ghana, Limpopo	Malaria, Tuberculosis
232	0	Campanulaceae	*Lobelia*	*Lobelia pyramidalis* Wall	Nepal	Nepal	Fever
233	0	Canellaceae	*Cinnamosma*	*Cinnamosma fragrans* H. Bn	Madagascar	Madagascar	Malaria
234	MN257823.1	Canellaceae	*Warburgia*	*Warburgia spp*	Multicultural	kenya, Uganda, Limpopo	Malaria, Tuberculosis
235	0	Canellaceae	*Warburgia*	*Warburgia salutaris* (G. Bertol.)	Limpopo	Limpopo	Tuberculosis
236	0	Canellaceae	*Warburgia*	*Warburgia ugandensis* Sprague	Uganda	Uganda	Tuberculosis
237	FJ939544.1	Cannaceae	*Canna*	*Canna indica* L.	Nigeria	Nigeria	Malaria
238	0	Capparaceae	*Capparis*	*Capparis zeylanica*	India	India	Diarrhea
239	0	Capparaceae	*Buchholzia*	*Buchholzia coriacea* Engl.	Nigeria	Nigeria	Fever
240	AY461547.1	Caricaceae	*Carica*	*Carica papaya* L	Multicultural	Northeast india, Uganda, Zimbabwe, Nigeria, Indonesia , Ghana	Malaria, Fever, Tuberculosis
241	0	Caryophilaceae	*Krauseola*	*Krauseola mossambicina*	Maputaland	Maputaland	Diarrhea
242	FJ980408.1	Caryophilaceae	*Drymaria*	*Drymaria cordata*	Nepal	Nepal	Fever
243	0	Celastraceae	*Celastrus*	*Celastrus indica*	Nigeria	Nigeria	Fever
244	0	Celastraceae	*Maytenus*	*Maytenus undata* (Thunb.) Blakelock	Kenya	Kenya	Malaria
245	KJ004285.1	Celastraceae	*Maytenus*	*Maytenus senegalensis* (Lam.) Exel/*Gymnosporia spp*	Multicultural	Kenya, Uganda, Maputaland, Limpopo	Malaria, Tuberculosis, Diarrhea
246	0	Celastraceae	*Elaeodendron*	*Elaeodendron transvaalense* (Burtt Davy) R.H.Archer	Limpopo	Limpopo	Tuberculosis
247	0	Celastraceae	*Gymnosporia*	*Gymnosporia maranguensis* (Loes.) Loe	Limpopo	Limpopo	Tuberculosis
248	0	Celastraceae	*Gymnosporia*	*Gymnosporia senegalensis* (Lam.) Loes	Limpopo	Limpopo	Tuberculosis
249	DQ217525.1	Celastraceae	*Pleurostylia*	*Pleurostylia capensis* (Turcz.) Loes	Limpopo	Limpopo	Tuberculosis
250	HM230141.1	Celastraceae	*Pristimera*	*Pristimera longipetiolata* (Oliv.) N.Hall	Limpopo	Limpopo	Tuberculosis
251	FJ980362.1	Chenopodiaceae	*Chenopodium*	*Chenopodium ambrosioides*	Maputaland	Maputaland	Diarrhea
252	EU128453.1	Clusiaceae	*Garcinia*	*Garcinia spp*	Multicultural	Nigeria, Maputaland	Malaria, Diarrhea
253	0	Clusiaceae	*Garcinia*	*Garcinia buchananii* Baker	Uganda	Uganda	Tuberculosis
254	0	Clusiaceae	*Garcinia*	*Garcinia gerrardii* Harv. ex Sim	Limpopo	Limpopo	Tuberculosis
255	KY670819.1	Cochlorspermaceae	*Cochlospermum*	*Cochlospermum tinctorium* A. Rich	Nigeria	Nigeria	Fever

230	MF963893.1	Caesalpiniaceae	*Senna*	*Senna spp.*	Multicultural	Uganda, Ivory coast, Ghana, Kenya, Nigeria, Iran, Zimbabwe, Limpopo, Maputaland, India	Malaria, Fever, Tuberculosis, Diarrhea
231	0	Caesalpiniaceae	*Cassia*	*Cassia occidentalis* Linn/*Senna occidentalis*	Multicultural	Ivory coast, Kenya, Ghana, Limpopo	Malaria, Tuberculosis
232	0	Campanulaceae	*Lobelia*	*Lobelia pyramidalis* Wall	Nepal	Nepal	Fever
233	0	Canellaceae	*Cinnamosma*	*Cinnamosma fragrans* H. Bn	Madagascar	Madagascar	Malaria
234	MN257823.1	Canellaceae	*Warburgia*	*Warburgia spp*	Multicultural	kenya, Uganda, Limpopo	Malaria, Tuberculosis
235	0	Canellaceae	*Warburgia*	*Warburgia salutaris* (G. Bertol.)	Limpopo	Limpopo	Tuberculosis
236	0	Canellaceae	*Warburgia*	*Warburgia ugandensis* Sprague	Uganda	Uganda	Tuberculosis
237	FJ939544.1	Cannaceae	*Canna*	*Canna indica* L.	Nigeria	Nigeria	Malaria
238	0	Capparaceae	*Capparis*	*Capparis zeylanica*	India	India	Diarrhea
239	0	Capparaceae	*Buchholzia*	*Buchholzia coriacea* Engl.	Nigeria	Nigeria	Fever
240	AY461547.1	Caricaceae	*Carica*	*Carica papaya* L	Multicultural	Northeast india, Uganda, Zimbabwe, Nigeria, Indonesia , Ghana	Malaria, Fever, Tuberculosis
241	0	Caryophilaceae	*Krauseola*	*Krauseola mossambicina*	Maputaland	Maputaland	Diarrhea
242	FJ980408.1	Caryophilaceae	*Drymaria*	*Drymaria cordata*	Nepal	Nepal	Fever
243	0	Celastraceae	*Celastrus*	*Celastrus indica*	Nigeria	Nigeria	Fever
244	0	Celastraceae	*Maytenus*	*Maytenus undata* (Thunb.) Blakelock	Kenya	Kenya	Malaria
245	KJ004285.1	Celastraceae	*Maytenus*	*Maytenus senegalensis* (Lam.) Exel/*Gymnosporia spp*	Multicultural	Kenya, Uganda, Maputaland, Limpopo	Malaria, Tuberculosis, Diarrhea
246	0	Celastraceae	*Elaeodendron*	*Elaeodendron transvaalense* (Burtt Davy) R.H.Archer	Limpopo	Limpopo	Tuberculosis
247	0	Celastraceae	*Gymnosporia*	*Gymnosporia maranguensis* (Loes.) Loe	Limpopo	Limpopo	Tuberculosis
248	0	Celastraceae	*Gymnosporia*	*Gymnosporia senegalensis* (Lam.) Loes	Limpopo	Limpopo	Tuberculosis
249	DQ217525.1	Celastraceae	*Pleurostylia*	*Pleurostylia capensis* (Turcz.) Loes	Limpopo	Limpopo	Tuberculosis
250	HM230141.1	Celastraceae	*Pristimera*	*Pristimera longipetiolata* (Oliv.) N.Hall	Limpopo	Limpopo	Tuberculosis
251	FJ980362.1	Chenopodiaceae	*Chenopodium*	*Chenopodium ambrosioides*	Maputaland	Maputaland	Diarrhea
252	EU128453.1	Clusiaceae	*Garcinia*	*Garcinia spp*	Multicultural	Nigeria, Maputaland	Malaria, Diarrhea
253	0	Clusiaceae	*Garcinia*	*Garcinia buchananii* Baker	Uganda	Uganda	Tuberculosis
254	0	Clusiaceae	*Garcinia*	*Garcinia gerrardii* Harv. ex Sim	Limpopo	Limpopo	Tuberculosis
255	KY670819.1	Cochlorspermaceae	*Cochlospermum*	*Cochlospermum tinctorium* A. Rich	Nigeria	Nigeria	Fever

256	0	Combretaceae	*Anogeissus*	*Anogeissus leiocarpus* (DC.) Guill & Perr	Nigeria	Nigeria	Fever
257	0	Combretaceae	*Combretum*	*Combretum illairii* Engl.	Kenya	Kenya	Malaria
258	0	Combretaceae	*Terminalia*	*Terminalia ivorensis* A. Chev	Ghana	Ghana	Malaria
259	0	Combretaceae	*Terminalia*	*Terminalia spinosa* Engl.	Kenya	Kenya	Malaria
260	EU338046.1	Combretaceae	*Combretum*	*Combretum spp*	Multicultural	Kenya, Uganda, Limpopo	Malaria, Tuberculosis
261	MH432182.1	Combretaceae	*Terminalia*	*Terminalia spp.*	Multicultural	Ghana, Limpopo, Maputaland, Meghalaya	Malaria, Tuberculosis, Diarrhea
262	0	Combretaceae	*Combretum*	*Combretum aculeatum* Vent	Senegal	Senegal	Tuberculosis
263	0	Combretaceae	*Combretum*	*Combretum hereroense*	Limpopo	Limpopo	Tuberculosis
264	0	Combretaceae	*Combretum*	*Combretum molle* R.Br. ex. G. Don	Uganda	Uganda	Tuberculosis
265	0	Combretaceae	*Combretum*	*Combretum paniculatum* Vent.	Senegal	Senegal	Tuberculosis
266	0	Combretaceae	*Terminalia*	*Terminalia sericea* Burch. ex DC.	Limpopo	Limpopo	Tuberculosis
267	FJ381769.1	Combretaceae	*Guiera*	*Guiera senegalensis* J.F. GMel	Multicultural	Senegal, Nigeria	Tuberculosis, Diarrhea
268	0	Commelinaceae	*Floscopa*	*Floscopa africana* (P.Beauv.) C.B.Clarke	Nigeria	Nigeria	Fever
269	0	Commelinaceae	*palisota*	*palisota hirsota*	Nigeria	Nigeria	Fever
270	0	Connaraceae	*Roureopsis*	*Roureopsis obliquifoliolata*	Congo	Congo	Diarrhea
271	KC528926.1	Convolvulaceae	*Convolvulus*	*Convolvulus scammonia* L	Iran	Iran	Fever
272	HQ728520.1	Convolvulaceae	*Cuscuta*	*Cuscuta reflexa*	India	India	Fever
273	MN824794.1	Convolvulaceae	*Ipomoea*	*Ipomoea albivenia* (Lindl.)	Limpopo	Limpopo	Tuberculosis
274	MH566959.1	Cornaceae	*Alangium*	*Alangium chinense*	Uganda	Uganda	Tuberculosis
275	MG730644.1	Crassulaceae	*Bryophyllum*	*Bryophyllum pinnatum*	Nigeria	Nigeria	Fever
276	0	Crassulaceae	*Rhodiola*	*Rhodiola imbricata* Edgew	Nepal	Nepal	Fever
277	KP114736.1	Crassulaceae	*Rhodiola*	*Rhodiola spp.*	Nepal	Nepal	Fever
278	0	Crassulaceae	*Kalanchoe*	*Kalanchoe glaucescens* Planch. ex benth	Uganda	Uganda	Tuberculosis
279	KJ026937.1	Cucurbitaceae	*Lagenaria*	*Lagenaria siceraria*	Nepal	Nepal	Diarrhea
280	0	Cucurbitaceae	*Coccinia*	*Coccinia cordifolia* (L.) Cogn.	Bangladesh	Bangladesh	Fever
281	GQ240881.1	Cucurbitaceae	*Trichosanthes*	*Trichosanthes dioica*	India	India	Fever
282	0	Cupresaceae	*Juniperus*	*Juniperus indica*	Nepal	Nepal	Diarrhea
283	AY283435.1	Cupresaceae	*Juniperus*	*Juniperus spp*	Nepal	Nepal	Fever, Diarrhea
284	GQ183047.1	Curcubitaceae	*Solena*	*Solena heterophylla* Lour	Nepal	Nepal	Fever
285	0	Curcubitaceae	*Gerranthus*	*Gerranthus lobatus* (Cogn.) Jeffrey	Kenya	Kenya	Malaria
286	0	Curcubitaceae	*Momordica*	*Momordica balsamina* L	Zimbabwe	Zimbabwe	Malaria

287	JN407450.1	Curcubitaceae	*Momordica*	*Momordica spp*	Multicultural	Indonesia , Ghana, Nigeria, Nepal, Zimbabwe	Malaria, Fever
288	0	Curcubitaceae	*Momordica*	*Momordica foetida* Schumach	Multicultural	Zimbabwe, Uganda	Malaria, Tuberculosis
289	AM981119.1	Curcubitaceae	*Cucumis*	*Cucumis spp*	Limpopo	Limpopo	Tuberculosis
290	0	Curcubitaceae	*Cucumis*	*Cucumis zeyheri* Sond	Limpopo	Limpopo	Tuberculosis
291	0	Cyperaceae	*Cyperus*	*Cyperus rotundus*	Congo	Congo	Diarrhea
292	MH768132.1	Cyperaceae	*Cyperus*	*Cyperus spp*	Multicultural	Indonesia, Bizana	Malaria, Diarrhea
293	0	Cyperaceae	*Cyperus*	*Cyperus sexangularis* Nees	Limpopo	Limpopo	Tuberculosis
294	AY096030.1	Dilleniaceae	*Dillenia*	*Dillenia indica* L.	Bangladesh	Bangladesh	Fever
295	0	Dioscoreaceae	*Dracaena*	*Dracaena loureiri* Gagnep	Thailand	Thailand	Fever
296	KJ956698.1	Dioscoreaceae	*Dioscorea*	*Dioscorea spp*	Multicultural	Nigeria, India	Fever, Diarrhea
297	0	Dioscoreaceae	*Dioscorea*	*Dioscorea dumetorum* Kunth	Nigeria	Nigeria	Malaria
298	0	Dioscoreaceae	*Dioscorea*	*Dioscorea dregeana* (Kunth)	Limpopo	Limpopo	Tuberculosis
299	0	Dioscoreaceae	*Dioscorea*	*Dioscorea sylvatica* Eckl. var. brevipes (Burtt Davy) Burkill	Limpopo	Limpopo	Tuberculosis
300	KM514673.1	Dipterocarpaceae	*Shorea*	*Shorea robusta*	India	India	Diarrhea
301	0	Dracaenaceae	*Sansevieria*	*Sansevieria hyacinthoides*	Limpopo	Limpopo	Diarrhea
302	0	Dracaenaceae	*Dracaena*	*Dracaena loureiri* Gagnep	Thailand	Thailand	Fever
303	0	Ebenaceae	*Diospyrosmes*	*Diospyrosmes pilliformis* Hochst. ex A.DC	Nigeria	Nigeria	Malaria
304	0	Ebenaceae	*Euclea*	*Euclea natalensis* A.DC	Zimbabwe	Zimbabwe	Malaria
305		Ebenaceae	*euclea*	*Euclea divinorum*	Zimbabwe	Zimbabwe	Malaria, Tuberculosis, Diarrhea
306	0	Ebenaceae	*Diospyros*	*Diospyros mespiliformis* Hochst. ex A.DC SSS36	Limpopo	Limpopo	Tuberculosis
307	0	Ebenaceae	*Euclea*	*Euclea undulata* Thunb	Limpopo	Limpopo	Tuberculosis
308	AY755760.1	Ephedraceae	*Ephedra*	*Ephedra gerardiana* Wall. ex Stapf	Nepal	Nepal	Fever
309	0	Ericaceae	*Gaultheria*	*Gaultheria fragantissima*	Meghalaya	Meghalaya	Diarrhea
310	0	Ericaceae	*Rhododendron*	*Rhododendron campanulatum* D.Don	Nepal	Nepal	Fever
311	MH710817.1	Euphorbiaceae	*Acalypha*	*Acalypha australis*	Bangladesh	Bangladesh	Diarrhea
312	0	Euphorbiaceae	*Clutia*	*Clutia pulchella*	Bizana	Bizana	Diarrhea
313	0	Euphorbiaceae	*Euphorbia*	*Euphorbia royleana*	Nepal	Nepal	Diarrhea
314	0	Euphorbiaceae	*Phyllanthus*	*Phyllanthus emblica*	Nepal	Nepal	Diarrhea
315	0	Euphorbiaceae	*Croton*	*Croton mubango*	Congo	Congo	Diarrhea
316	0	Euphorbiaceae	*Emblica*	*Emblica officinalis*	India	India	Fever

317	0	Euphorbiaceae	*Croton*	*Croton caudatus* Geisel	Northeast india	Northeast india	Malaria
318	MN257852.1	Euphorbiaceae	*Flueggea*	*Flueggea virosa* (Willd.) Voigt	Multicultural	Kenya ,Ghana	Malaria
319	0	Euphorbiaceae	*Homonoia*	*Homonoia riparia* Lour	Northeast india	Northeast india	Malaria
320	KF500512.1	Euphorbiaceae	*Jatropha*	*Jatropha curcas* L.	Multicultural	Uganda, india	Malaria
321	DQ866581.1	Euphorbiaceae	*Macaranga*	*Macaranga schweinfurthii* Pax	Uganda	Uganda	Malaria
322	DQ866606.1	Euphorbiaceae	*Mallotus*	*Mallotus oppositifolius* Mull. Arg	Nigeria	Nigeria	Malaria
323	0	Euphorbiaceae	*Mareya*	*Mareya micrantha*	Ivory coast	Ivory coast	Malaria
324	0	Euphorbiaceae	*Phyllanthus*	*Phyllanthus (pseudo) niruri* Mull. Arg.	Uganda	Uganda	Malaria
325	0	Euphorbiaceae	*Phyllanthus*	*Phyllanthus muellerianus* (Kuntze)	Ivory coast	Ivory coast	Malaria
326	0	Euphorbiaceae	*Shirakiopsis*	*Shirakiopsis elliptica* (Hochst.) H.–J. Esser	Uganda	Uganda	Malaria
327	0	Euphorbiaceae	*Suregada*	*Suregada zanzibarensis* Baill	Kenya	Kenya	Malaria
328	0	Euphorbiaceae	*Tetrorchidium*	*Tetrorchidium didymostemon* (Baill.) Pax & K. Hoffm.	Uganda	Uganda	Malaria
329	MH768145.1	Euphorbiaceae	*Euphorbia*	*Euphorbia spp*	Multicultural	Ivory coast, Nigeria, Senegal, Nepal	Malaria, Fever, Tuberculosis, Diarrhea
330	KY968910.1	Euphorbiaceae	*Phyllanthus*	*Phyllanthus spp.*	Multicultural	Ghana , Nigeria, Uganda, Ivory coast, Nepal	Malaria, Fever, Tuberculosis, Diarrhea
331	0	Euphorbiaceae	*Alchornea*	*Alchornea cordifolia* (Schumach. and Thonn.)	Multicultural	Ivory coast, Uganda	Malaria, Tuberculosis
332	MH768161.1	Euphorbiaceae	*Ricinus*	*Ricinus communis* L.	Multicultural	Kenya, Limpopo	Malaria, Tuberculosis
333	KP092923.1	Euphorbiaceae	*Croton*	*Croton spp*	Multicultural	Northeast india, Limpopo, Congo	Malaria, Tuberculosis, Diarrhea
334	FJ439920.1	Euphorbiaceae	*Bridelia*	*Bridelia micrantha* (Hochst.) Baill	Uganda	Uganda	Tuberculosis
335	0	Euphorbiaceae	*Croton*	*Croton gratissimus* Burch	Limpopo	Limpopo	Tuberculosis
336	0	Euphorbiaceae	*Croton*	*Croton menyharthii* Pax	Limpopo	Limpopo	Tuberculosis
337	0	Euphorbiaceae	*Jatropha*	*Jatropha zeyheri*	Limpopo	Limpopo	Tuberculosis
338	0	Euphorbiaceae	*Sapium*	*Sapium ellipticum* (Hochst.) Pax	Uganda	Uganda	Tuberculosis
339	KP794335.1	Euphorbiaceae	*Tragia*	*Tragia dioica*	Limpopo	Limpopo	Tuberculosis
340	0	Fabaceae	*Albizia*	*Albizia antunesiana*	Zimbabwe	Zimbabwe	Diarrhea
341	0	Fabaceae	*Alhagi*	*Alhagi pseudalhagi*	Iran	Iran	Diarrhea
342	KX057832.1	Fabaceae	*Alysicarpus*	*Alysicarpus rugosus*	Bizana	Bizana	Diarrhea
343	0	Fabaceae	*Peltophorum*	*Peltophorum africanum*	Zimbabwe	Zimbabwe	Diarrhea
344	AY748439.1	Fabaceae	*Vigna*	*Vigna unguiculata*	Limpopo	Limpopo	Diarrhea

345	KJ436384.1	Fabaceae	*Butea*	*Butea frondosa/ Butea monosperma*	India	India	Diarrhea
346	AF189023.1	Fabaceae	*Dalbergia*	*Dalbergia sissoo*	India	India	Diarrhea
347	MH768281.1	Fabaceae	*Desmodium*	*Desmodium triflorum*	India	India	Diarrhea
348	0	Fabaceae	*Pongamia*	*Pongamia glabra*	India	India	Diarrhea
349	AF467493.1	Fabaceae	*Pongamia*	*Pongamia spp*	India	India	Diarrhea
350	0	Fabaceae	*Sesbania*	*Sesbania grandifolia*	India	India	Diarrhea
351	0	Fabaceae	*Sophora*	*Sophora mollis*	Nigeria	Nigeria	Diarrhea
352	0	Fabaceae	*Cassia*	*Cassia angustifolia* Vahl/*Senna alexandrina*	Iran	Iran	Fever
353	MH260279.1	Fabaceae	*Clitoria*	*Clitoria ternatea*	India	India	Fever
354	KY968966.1	Fabaceae	*Melilotus*	*Melilotus officinalis* Linn.	Nepal	Nepal	Fever
355	JX494756.1	Fabaceae	*Alhagi*	*Alhagi spp*	Iran	Iran	Fever, Diarrhea
356	0	Fabaceae	*Albizia*	*Albizia ferruginea* (Guill. and Perr.)	Ivory coast	Ivory coast	Malaria
357	0	Fabaceae	*Cassia*	*Cassia sieberiana* DC	Ivory coast	Ivory coast	Malaria
358	0	Fabaceae	*Crotalaria*	*Crotalaria ochroleuca* G. Don	Northeast india	Northeast india	Malaria
359	0	Fabaceae	*Crotolaria*	*Crotolaria occulta* Grab	Northeast india	Northeast india	Malaria
360	0	Fabaceae	*Entada*	*Entada africana* Guill. & Perr.	Northeast india	Northeast india	Malaria
361	0	Fabaceae	*Erythrina*	*Erythrina excelsa* Bak	Northeast india	Northeast india	Malaria
362	0	Fabaceae	*Millettia*	*Millettia zechiana* Harms	Ivory coast	Ivory coast	Malaria
363	0	Fabaceae	*Tetrapleura*	*Tetrapleura tetraptera*	Ghana	Ghana	Malaria
364	AF467015.1	Fabaceae	*Abrus*	*Abrus precatorius* L.	Kenya	Kenya	Malaria, Fever
365	KX057842.1	Fabaceae	*Cajanus*	*Cajanus cajan* (L.) Druse	Multicultural	Northeast india, Nigeria	Malaria, Fever
366	MK207321.1	Fabaceae	*Erythrina*	*Erythrina spp.*	Multicultural	Northeast india, Uganda, Limpopo	Malaria, Fever, Tuberculosis
367	MN852274.1	Fabaceae	*Indigofera*	*Indigofera sp.* (GOM 1)	Multicultural	Ghana, Uganda	Malaria, Tuberculosis
368	JQ067205.1	Fabaceae	*Crotalaria*	*Crotalaria spp*	Multicultural	Northeast india, Uganda	Malaria, Tuberculosis
369	KX057869.1	Fabaceae	*Entada*	*Entada abyssinica* Steud. ex A. Rich.	Multicultural	Northeast india, Uganda	Malaria, Tuberculosis
370	0	Fabaceae	*Acacia*	*Acacia erioloba* E.Mey./*Vachellia erioloba*	Limpopo	Limpopo	Tuberculosis
371	0	Fabaceae	*Acacia*	*Acacia hockii* De Wild	Uganda	Uganda	Tuberculosis
372	0	Fabaceae	*Acacia*	*Acacia spectabilis*	Uganda	Uganda	Tuberculosis
373	0	Fabaceae	*Albizia*	*Albizia adianthifolia* (Schumach.) W.Wight *var. adianthifolia*	Limpopo	Limpopo	Tuberculosis
374	0	Fabaceae	*Albizia*	*Albizia coriaria* Welw.ex Oliv	Uganda	Uganda	Tuberculosis
375	KX057840.1	Fabaceae	*Burkea*	*Burkea africana* Hook	Limpopo	Limpopo	Tuberculosis
376	0	Fabaceae	*Desmodium*	*Desmodium salicifolium* (Poir.) D.C.	Uganda	Uganda	Tuberculosis
377	0	Fabaceae	*Detarium*	*Detarium senegalense* J.F.Gmel.	Senegal	Senegal	Tuberculosis

378	0	Fabaceae	*Elephantorrhiza*	*Elephantorrhiza burkei* Benth	Limpopo	Limpopo	Tuberculosis
379	0	Fabaceae	*Erythrina*	*Erythrina lysistemon* Hutch	Limpopo	Limpopo	Tuberculosis
380	0	Fabaceae	*Indigofera*	*Indigofera emarginella* Steud. ex A. Rich	Uganda	Uganda	Tuberculosis
381	EU720492.1	Fabaceae	*Lepisanthes*	*Lepisanthes senegalensis* (Poir.) Leehn	Senegal	Senegal	Tuberculosis
382	AF467482.1	Fabaceae	*Mundulea*	*Mundulea sericea* Willd	Limpopo	Limpopo	Tuberculosis
383	0	Fabaceae	*Parkia*	*Parkia filicoidea* Welw. ex Oliv	Uganda	Uganda	Tuberculosis
384	FJ172178.1	Fabaceae	*Phaseolus*	*Phaseolus vulgaris* L.	Uganda	Uganda	Tuberculosis
385	0	Fabaceae	*Piptadeniastrum*	*Piptadeniastrum africanum* (Hook. f.) Brenan	Uganda	Uganda	Tuberculosis
386	JN083479.1	Fabaceae	*Pterocarpus*	*Pterocarpus erinaceus* Poir	Senegal	Senegal	Tuberculosis
387	0	Fabaceae	*Rhynchosia*	*Rhynchosia hirta* (Andrews)	Limpopo	Limpopo	Tuberculosis
388	0	Fabaceae	*Senna*	*Senna italica* Mill. subsp. *arachoides* (Burch.) Lock	Limpopo	Limpopo	Tuberculosis
389	0	Fabaceae	*Senna*	*Senna petersiana* (Bolle) Lock	Limpopo	Limpopo	Tuberculosis
390	0	Fabaceae	*Tylosema*	*Tylosema fassoglense* (Schweinf.) Torre & Hillc	Limpopo	Limpopo	Tuberculosis
391	KX057868.1	Fabaceae	*Dichrostachys*	*Dichrostachys cinerea* (L.)	Multicultural	Limpopo, India	Tuberculosis, Diarrhea
392	MN992833.1	Fabaceae	*Schotia*	*Schotia brachypetala* Sond	Multicultural	Limpopo, Maputaland	Tuberculosis, Diarrhea
393	0	Fagaceae	*Quercus*	*Quercus brantii*	Iran	Iran	Diarrhea
394	KX838252.1	fagaceae	*Quercus*	*Quercus spp*	Multicultural	Northeast India	Fever, Diarrhea
395	0	Flacourtiaceae	*Gynocardia*	*Gynocardia odorata*	Nepal	Nepal	Fever
396	DQ521289.1	Flacourtiaceae	*Flacourtia*	*Flacourtia indica* (Burm.f) Merr.	Multicultural	Kenya, Zimbabwe	Malaria, Diarrhea
397	0	Fumariaceae	*Corydalis*	*Corydalis govaniana* Wall	Nepal	Nepal	Fever
398	KX282162.1	Fumariaceae	*Fumaria*	*Fumaria parviflora* Lam	Iran	Iran	Fever
399	KU512311.2	Gentianaceae	*Gentiana*	*Gentiana algida* Pallas	Nepal	Nepal	Fever
400	AJ294675.1	Gentianaceae	*Gentianella*	*Gentianella moorcroftiana* (Wall. ex Griseb.) Airy Shaw	Nepal	Nepal	Fever
401	0	Gentianaceae	*Swertia*	*Swertia petiolata* Royle ex D. Don	Nepal	Nepal	Fever
402	JX569818.1	Gentianaceae	*Swertia*	*Swertia spp*	Multicultural	Nepal, Northeast india	Malaria, Fever
403	MN561250.1	Gentinaceae	*Halenia*	*Halenia elliptica* D. Don	Northeast india	Northeast india	Malaria
404	0	Gentinaceae	*Swertia*	*Swertia dilatata* Wall.	Northeast india	Northeast india	Malaria
405	0	Gentinaceae	*Swertia*	*Swertia nervosa* Wall	Northeast india	Northeast india	Malaria
406	FJ232582.1	Gentinaceae	*Enicostema*	*Enicostema axillare* (Lam.) A.	Limpopo	Limpopo	Tuberculosis
407	AF256560.1	Geraniaceae	*Pelargonium*	*Pelargonium luridum*	Bizana	Bizana	Diarrhea
408	0	Geraniaceae	*Geranium*	*Geranium pretense* Linn	Nepal	Nepal	Fever
409	AF349295.1	Gesneriaceae	*Aeschynanthus*	*Aeschynanthus sikkimensis* Stapf	Nepal	Nepal	Fever
410	0	Gramineae	*Andropogon*	*Andropogon schœnanthus/nardis* L.	Madagascar	Madagascar	Malaria

411	AF426350.1	Grossulariaceae	*Ribes*	*Ribes uva-crispa* L.	Uganda	Uganda	Tuberculosis
412	0	Hyacinthaceae	*Ledebouria*	*Ledebouria ovatifolia*	Bizana	Bizana	Diarrhea
413	0	Hyacinthaceae	*Drimia*	*Drimia elata* Jacq.	Limpopo	Limpopo	Tuberculosis
414	0	Hyacinthaceae	*Drimia*	*Drimia sanguinea* (Schinz)Jessop	Limpopo	Limpopo	Tuberculosis
415	0	Hyacinthaceae	*Eucomis*	*Eucomis autumnalis* (Mill.)	Multicultural	Limpopo, Bizana	Tuberculosis, Diarrhea
416	0	Hydnoraceae	*Hydnora*	*Hydnora africana*	Bizana	Bizana	Diarrhea
417	KP092583.1	Hydrangiaceae	*Dichroa*	*Dichroa febrifuga* Lour	Multicultural	nepal, northeast india	Malaria, Fever
418	MN999720.1	Hypericaceae	*Psorospermum*	*Psorospermum febrifugum* Spach.	Nigeria	Nigeria	Fever
419	KC709362.1	Hypericaceae	*Harungana*	*Harungana madagascariensis* Poir.	Multicultural	kenya, nigeria	Malaria, Fever
420	0	Hypoxidaceae	*Curculigo*	*Curculigo orchioides*	Nepal	Nepal	Diarrhea
421	0	Hypoxidaceae	*Hypoxis*	*Hypoxis hemerocallidea*	Bizana	Bizana	Diarrhea
422	0	Hypoxidaceae	*Curculigo*	*Curculigo pilosa* Schum & Thonn	Nigeria	Nigeria	Fever
423	0	Icacinaceae	*Cassinopsis*	*Cassinopsis ilicifolia* (Hochst.)Kuntze	Limpopo	Limpopo	Tuberculosis
424	0	Icacinaceae	*Pyrenacantha*	*Pyrenacantha grandiflora* Baill	Limpopo	Limpopo	Tuberculosis
425	0	Kirkiaceae	*Kirkia*	*Kirkia wilmsii* Engl.	Limpopo	Limpopo	Tuberculosis
426	0	Labiatae	*Ocimum*	*Ocimum basilicum* L.	Kenya	Kenya	Malaria
427	0	Labietaceae	*Hoslundia*	*Hoslundia opposite* Vahl	Kenya	Kenya	Malaria
428	0	Lamiaceae	*Thymus*	*Thymus linearis*	Nepal	Nepal	Diarrhea
429	0	Lamiaceae	*Ajuga*	*Ajuga integrifolia*	pakistan	pakistan	Fever
430	KM877374.1	Lamiaceae	*Coleus*	*Coleus amboinicus/Plectranthus amboinicus*	India	India	Fever
431	MK881159.1	Lamiaceae	*Leucas*	*Leucas aspera*	India	India	Fever
432	0	Lamiaceae	*Leucas*	*Leucas lavendulifolia* Sm.	Bangladesh	Bangladesh	Fever
433	0	Lamiaceae	*Melissa*	*Melissa parviflora* Benth	Nepal	Nepal	Fever
434	0	Lamiaceae	*Nepeta*	*Nepeta discolor* Royle ex Benth	Nepal	Nepal	Fever
435	0	Lamiaceae	*Nepeta*	*Nepeta floccosa* Benth	Nepal	Nepal	Fever
436	KC535539.1	Lamiaceae	*Scutellaria*	*Scutellaria discolor* Colebr	Nepal	Nepal	Fever
437	KF769031.1	Lamiaceae	*Stachys*	*Stachys spp*	Multicultural	Nepal, Limpopo	Fever, Tuberculosis
438	KT210237.1	Lamiaceae	*Elsholtzia*	*Elsholtzia blanda* Benth	Northeast india	Northeast india	Malaria
439	0	Lamiaceae	*Gomphostemma*	*Gomphostemma parviflora* Wall.	Northeast india	Northeast india	Malaria
440	0	Lamiaceae	*Mesona*	*Mesona wallichiana* Benth	Northeast india	Northeast india	Malaria
441	0	Lamiaceae	*Ocimum*	*Ocimum angustifolium* Benth.	Zimbabwe	Zimbabwe	Malaria
442	0	Lamiaceae	*Ocimum*	*Ocimum gratissimum* L.	Multicultural	Ghana, Nigeria	Malaria, Fever
443	MF468205.1	Lamiaceae	*Ocimum*	*Ocimum spp.*	Multicultural	Northeast india, Bangladesh, Ghana, Nigeria, Kenya	Malaria, Fever
444	0	Lamiaceae	*Clerodendrum*	*Clerodendrum ternatum* Schinz	Limpopo	Limpopo	Tuberculosis
445	JF301584.1	Lamiaceae	*Hyptis*	*Hyptis suaveolens* (L.) Poit	Uganda	Uganda	Tuberculosis
446	0	Lamiaceae	*Stachys*	*Stachys spp*	Limpopo	Limpopo	Tuberculosis
447	0	Lamiaceae	*Tetradenia*	*Tetradenia riparia* (Hochst.) Codd	Uganda	Uganda	Tuberculosis
448	0	Lauraceae	*Cinnamomum*	*Cinnamomum pauciflorum*	Meghalaya	Meghalaya	Diarrhea
449	0	Lauraceae	*Cinnamomum*	*Cinnamomum tamala*	Multicultural	meghalaya, nepal	Diarrhea
450	AF272298.1	Lauraceae	*Ocotea*	*Ocotea bullata*	Bizana	Bizana	Diarrhea
451	0	Lauraceae	*Cinnamonum*	*Cinnamonum bejolghota* (Buch.-Ham)	Northeast india	Northeast india	Malaria
452	KX509877.1	Lauraceae	*Persea*	*Persea americana* Mill	Multicultural	Nigeria, Uganda	Malaria, Fever, Tuberculosis

453	KX766399.1	Lauraceae	*Cinnamomum*	*Cinnamomum zeylanicum Blume/Cinnamomum spp*	Multicultural	Uganda, Meghalaya	Tuberculosis, Diarrhea
454	0	Lecythidaceae	*Petersianthus*	*Petersianthus macrocarpus* (P.Beauv.)Liben	Ghana	Ghana	Malaria
455	0	Leguminosae	*Cassia*	*Cassia fistula*	India	India	Diarrhea
456	0	Leguminosae	*Pentacletra*	*Pentacletra macrophylla*	Nigeria	Nigeria	Fever
457	0	Leguminosae	*Acacia*	*Acacia nilotica* (L.)	Kenya	Kenya	Malaria
458	0	Leguminosae	*Cassia*	*Cassia abbreviata* Oliv	Multicultural	Zimbabwe, Kenya	Malaria
459	0	Leguminosae	*Desmodium*	*Desmodium mauritianum* D.C.	Madagascar	Madagascar	Malaria
460	MN257791.1	Leguminosae	*Albizia*	*Albizia spp*	Multicultural	Kenya, Zimbabwe	Malaria, Diarrhea
461	0	Liliaceae	*Asparagus*	*Asparagus racemosus*	Nepal	Nepal	Diarrhea
462	0	Liliaceae	*Protoasparagus*	*Protoasparagus racemosus*	India	India	Diarrhea
463	0	Liliaceae	*Allium*	*Allium ascalonicum* L. Backer	Nigeria	Nigeria	Fever
464	GQ184575.1	Liliaceae	*Paris*	*Paris polyphylla* Smith	Nepal	Nepal	Fever
465	JQ230982.1	Liliaceae	*Asparagus*	*Asparagus spp*	Multicultural	India, Zimbabwe, Nepal	Fever, Diarrhea
466	0	Liliaceae	*Aloe*	*Aloe deserti* Berger.	Kenya	Kenya	Malaria
467	0	Liliaceae	*Aloe*	*Aloe macrosiphon* Bak.	Kenya	Kenya	Malaria
468	0	Liliaceae	*Allium*	*Allium sativum* L	Multicultural	Nigeria, Uganda	Malaria, Fever, Tuberculosis
469	0	Loganiaceae	*Anthocleista*	*Anthocleista djalonensis* A. Chevalier	Ivory coast	Ivory coast	Malaria
470	0	Loganiaceae	*Anthocleista*	*Anthocleista nobilis* G. Don	Ghana	Ghana	Malaria
471	FJ232578.1	Loganiaceae	*Fagraea*	*Fagraea racemosa* Jack	Indonesia	Indonesia	Malaria
472	MN257683.1	Loganiaceae	*Strychnos*	*Strychnos spp*	Multicultural	Ivory coast, Maputaland	Malaria, Diarrhea
473	JQ740193.1	Lythraceae	*Punica*	*Punica granatum*	Multicultural	Limpopo, India	Diarrhea
474	AF420217.1	Lythraceae	*Lawsonia*	*Lawsonia inermis* L.	Multicultural	Nigeria, Senegal	Fever, Tuberculosis
475	EU593550.1	Magnoliacae	*Magnolia*	*Magnolia grandiflora* L	Northeast india	Northeast india	Malaria
476	0	Malpighiaceae	*Tristellateia*	*Tristellateia madagascariensis* Poir	Madagascar	Madagascar	Malaria
477	MH768225.1	Malvaceae	*Abutilon*	*Abutilon indicum*	Bangladesh	Bangladesh	Diarrhea
478	LC093518.1	Malvaceae	*Byttneria*	*Byttneria herbacea*	West bengal	West bengal	Diarrhea
479	0	Malvaceae	*Grewia*	*Grewia sapida*	West bengal	West bengal	Diarrhea
480	FJ204691.1	Malvaceae	*Malva*	*Malva parviflora*	Bizana	Bizana	Diarrhea
481	0	Malvaceae	*Triumffeta*	*Triumffeta spp*	Limpopo	Limpopo	Diarrhea
482	KP222461.1	Malvaceae	*Abelmoschus*	*Abelmoschus esculentus*	India	India	Fever
483	0	malvaceae	*Abutilon*	*Abutilon galpinii* A.Meeuse	Limpopo	Limpopo	Tuberculosis
484	U12713.1	malvaceae	*Gossypium*	*Gossypium herbaceum*	Limpopo	Limpopo	Tuberculosis
485	0	malvaceae	*Grewia*	*Grewia flava* DC.	Limpopo	Limpopo	Tuberculosis
486	0	malvaceae	*Grewia*	*Grewia occidentalis* L	Limpopo	Limpopo	Tuberculosis
487	0	malvaceae	*Hibiscus*	*Hibiscus fuscus* Garcke	Uganda	Uganda	Tuberculosis
488	0	Malvaceae	*Triumfetta*	*Triumfetta flavescens* Hochst. ex A. Rich	Uganda	Uganda	Tuberculosis
489	MH768331.1	Malvaceae	*Waltheria*	*Waltheria indica* L	Limpopo	Limpopo	Tuberculosis
490	MN177142.1	malvaceae	*Grewia*	*Grewia spp*	Multicultural	Limpopo, Zimbabwe, West bengal	Tuberculosis, Diarrhea
491	KY798015.1	Melastomaceae	*Melastoma*	*Melastoma malabathricum*	Bangladesh	Bangladesh	Diarrhea
492	0	Melastomaceae	*Osbeckia*	*Osbeckia crinata*	Meghalaya	Meghalaya	Diarrhea
493	0	Meliaceae	*Trichilia*	*Trichilia emetica*	Maputaland	Maputaland	Diarrhea
494	0	Meliaceae	*Turraea*	*Turraea obtusifolia*	Bizana	Bizana	Diarrhea

495	JN565010.1	Meliaceae	*Entandrophragma*	*Entandrophragma cylindricum*	Nigeria	Nigeria	Fever
496	FJ518899.1	Meliaceae	*Lovoa*	*Lovoa trichilioides*	Nigeria	Nigeria	Fever
497	KR364563.1	Meliaceae	*Trichilia*	*Trichilia monadelpha* (Thonn.)J.de Wilde	Ghana	Ghana	Malaria
498	KF840425.1	Meliaceae	*Khaya*	*Khaya spp.*	Nigeria	Nigeria	Malaria, Fever
499	AY695587.1	Meliaceae	*Lansium*	*Lansium domesticum* Corr. Serr	Indonesia	Indonesia	Malaria, Fever
500	AY695594.1	Meliaceae	*Azadirachta*	*Azadirachta indica* (A Juss)	Multicultural	Nigeria, Kenya , Ghana, Kenya, Uganda, Meghalaya, West bengal	Malaria, Fever, Tuberculosis, Diarrhea
501	0	Meliaceae	*Trichilia*	*Trichilia dregeana* Sond	Uganda	Uganda	Tuberculosis
502	AY695595.1	Meliaceae	*Melia*	*Melia azedarach* L	Multicultural	Limpopo, Maputaland	Tuberculosis, Diarrhea
503	MT137493.1	Melianthaceae	*Bersama*	*Bersama abyssinica* Fresen.	Ivory coast	Ivory coast	Malaria
504	0	Menispermaceae	*Epinetrum*	*Epinetrum villosum*	Congo	Congo	Diarrhea
505	0	Menispermaceae	*Cocculus*	*Cocculus cordifolia*	India	India	Fever
506	0	Menispermaceae	*Stephania*	*Stephania glabra*	Nepal	Nepal	Fever
507	MK256960.1	Menispermaceae	*Tinospora*	*Tinospora spp.*	Nepal	Nepal	Fever
508		Menispermaceae	*cocculus*	*Cocculus hirsutus*	India	India	Fever, Diarrhea
509	0	Menispermaceae	*Tinospora*	*Tinospora cordifolia* Willd	Multicultural	Nepal, India	Fever, Diarrhea
510	0	Menispermaceae	*Cissampelos*	*Cissampelos mucronate* A. Rich.	Kenya	Kenya	Malaria
511	KY365655.1	Menispermaceae	*Rhigiocarya*	*Rhigiocarya racemifera* Miers	Ivory coast	Ivory coast	Malaria
512	KJ566142.1	Menispermaceae	*Stephania*	*Stephania japonica* Miers.	Northeast india	Northeast india	Malaria
513	MK256959.1	Menispermaceae	*Cissampelos*	*Cissampelos spp*	Multicultural	Northeast india, Maputaland	Malaria, Diarrhea
514	KY365656.1	Menispermaceae	*Sphenocentrum*	*Sphenocentrum jollyanum* Pierre	Nigeria	Nigeria	Malaria, Fever
515	0	Mesembryanthemaceae	*Carpobrotus*	*Carpobrotus edulis* (L.) L.Bolus	Limpopo	Limpopo	Tuberculosis
516	KX057889.1	Mimosaceae	*Mimosa*	*Mimosa pudica* L.	Bangladesh	Bangladesh	Fever
517	0	Mimosaceae	*Parkia*	*Parkia biglobosa* (Jacq.) R. Br	Nigeria	Nigeria	Fever
518	0	Mimosaceae	*Acacia*	*Acacia senegal* (L.) Willd	Multicultural	Nigeria, Limpopo	Fever, Tuberculosis
519	AF360728.1	Mimosaceae	*Acacia*	*Acacia spp*	Multicultural	Northeast india, Limpopo, Uganda, Maputaland, Bizana	Malaria, Fever, Tuberculosis, Diarrhea
520	KT907365.1	Molluginaceae	*Mollugo*	*Mollugo nudicaulis*	India	India	Fever
521	KT207487.1	Moraceae	*Streblus*	*Streblus asper*	Bangladesh	Bangladesh	Diarrhea
522	MT012131.1	Moraceae	*Artocarpus*	*Artocarpus altilis*	Nigeria	Nigeria	Fever
523	0	Moraceae	*Ficus*	*Ficus capensis* Thunb	Ivory coast	Ivory coast	Malaria
524	0	Moraceae	*Ficus*	*Ficus exasperate*	Nigeria	Nigeria	Malaria
525	0	Moraceae	*Ficus*	*Ficus megapoda* Bak.	Madagascar	Madagascar	Malaria
526	0	Moraceae	*Melicia*	*Melicia excelsa* (Welw.)	Multicultural	Nigeria, Uganda	Malaria, Tuberculosis
527	EU091599.1	Moraceae	*Ficus*	*Ficus spp*	Multicultural	Kenya, Uganda, Limpopo, Senegal, Bizana	Malaria, Tuberculosis, Diarrhea
528	KT002559.1	Moraceae	*Antiaris*	*Antiaris toxicaria* Lesch.	Uganda	Uganda	Tuberculosis
529	0	Moraceae	*Ficus*	*Ficus abutilifolia* (Miq.) Miq.	Limpopo	Limpopo	Tuberculosis
530	0	Moraceae	*Ficus*	*Ficus glumosa* Delile	Uganda	Uganda	Tuberculosis
531	0	Moraceae	*Ficus*	*Ficus ingens* (Miq.) Miq	Limpopo	Limpopo	Tuberculosis

532	0	Moraceae	*Ficus*	*Ficus natalensis* Hochst	Uganda	Uganda	Tuberculosis
533	0	Moraceae	*Ficus*	*Ficus thonningii* Blume	Senegal	Senegal	Tuberculosis
534	0	Myricaceae	*Myrica*	*Myrica kandtiana* Engl.	Uganda	Uganda	Tuberculosis
535	0	myricaceae	*Myrica*	*Myrica spp*	Multicultural	Uganda, Meghalaya	Tuberculosis, Diarrhea
536	KP406145.1	Myristicaceae	*Myristica*	*Myristica fragrans*	Nepal	Nepal	Diarrhea
537	0	Myristicaceae	*Pycnatus*	*Pycnatus kombo*	Nigeria	Nigeria	Fever
538	0	Myristicaceae	*Ficus*	*Ficus capensis* Thunb	Ivory coast	Ivory coast	Malaria
539	0	Myristicaceae	*Pycnanthus*	*Pycnanthus angolensis* (Welw.)Warb.	multicultural	Ghana, Uganda	Malaria, Tuberculosis
540	0	Myrothamnaceae	*Myrothamnus*	*Myrothamnus flabellifolius Welw.*	Limpopo	Limpopo	Tuberculosis
541	0	Myrsinaceae	*Maesa*	*Maesa lanceolata*	Bizana	Bizana	Diarrhea
542	0	Myrtaceae	*Syzygium*	*Syzygium rubicundum*	India	India	Diarrhea
543	HM596038.1	Myrtaceae	*Eucalyptus*	*Eucalyptus camaldulensis* Dehnh	Multicultural	Nigeria, Limpopo, Bizana	Fever,Tuberculosis, Diarrhea
544	EF026622.1	Myrtaceae	*Syzygium*	*Syzygium spp*	Multicultural	Nigeria, Uganda, Maputaland, India	Fever,Tuberculosis, Diarrhea
545	AY487283.1	Myrtaceae	*Psidium*	*Psidium guajava* L	Multicultural	Nigeria, Ghana, Maputaland	Malaria, Fever, Diarrhea
546	KM064881.1	Myrtaceae	*Callistemon*	*Callistemon citrinus* (Curtis) Skeels	Uganda	Uganda	Tuberculosis
547	0	Myrtaceae	*Syzygium*	*Syzygium cumini* (L.) Skeels	Uganda	Uganda	Tuberculosis
548	0	Myrtaceae	*Syzygium*	*Syzygium gerrardii*	Limpopo	Limpopo	Tuberculosis
549	0	Nyctaginaceae	*Boerhaavia*	*Boerhaavia diffusa*	Nigeria	Nigeria	Fever
550	MK452746.1	Nymphaeaceae	*Nymphaea*	*Nymphaea lotus* L	Madagascar	Madagascar	Malaria
551	0	Ochnaceae	*Ochna*	*Ochna pulchra* Hook.	Limpopo	Limpopo	Tuberculosis
552	MK683227.1	Olacaceae	*Borassus*	*Borassus aethiopum*	Ghana	Ghana	Fever
553	0	Olacaceae	*Olax*	*Olax subscorpiodea*	Nigeria	Nigeria	Fever
554	KY780582.1	Olacaceae	*Olea*	*Olea europaea* L	Limpopo	Limpopo	Tuberculosis
555	MN257848.1	Olacaceae	*Ximenia*	*Ximenia spp*	Limpopo	Limpopo	Tuberculosis, Diarrhea
556	JX856583.1	Oleaceae	*Nyctanthes*	*Nyctanthes arbor-tristis*	West bengal	West bengal	Diarrhea
557	FM208221.1	Oleaceae	*Osmanthus*	*Osmanthus fragrans* Lour	Nepal	Nepal	Fever
558	KX168366.1	Onagraceae	*Ludwigia*	*Ludwigia peruviana* (L.) Hara	Nigeria	Nigeria	Malaria
559	MH768271.1	Oxalidaceae	*Oxalis*	*Oxalis corniculata*	Multicultural	meghalaya, nepal	Diarrhea
560	0	Palmae	*Bridelia*	*Bridelia ferruginea*	Ghana	Ghana	Fever
561	0	Pandaceae	*Microdesmis*	*Microdesmis keayana* J. Leonard	Ivory coast	Ivory coast	Malaria
562	MH768272.1	Papaveraceae	*Argemone*	*Argemone mexicana* L.	Multicultural	Nigeria, Uganda, Limpopo	Fever, Tuberculosis
563	GQ889049.1	Papilionaceae	*Securidaca*	*Securidaca longepedunculata* Fres.	Multicultural	Kenya, Nigeria, Limpopo	Malaria, Fever, Tuberculosis
564	0	Pasifloraceae	*Passiflora*	*Passiflora nepalensis* Walp	Multicultural	Northeast india, Nepal	Malaria, Fever
565	0	Pasifloraceae	*Adenia*	*Adenia fruticose* Burtt Davy	Limpopo	Limpopo	Tuberculosis
566	DQ521349.1	Pasifloraceae	*Adenia*	*Adenia spinosa* Burtt Davy	Limpopo	Limpopo	Tuberculosis
567	0	Pedaliaceae	*Dicerocaryum*	*Dicerocaryum senecioides* (Klotzsch)	Limpopo	Limpopo	Tuberculosis
568	0	Phyllanthaceae	*Phyllanthus*	*Phyllanthus reticulatus* Poir	Uganda	Uganda	Tuberculosis

569	EU196128.1	Pinaceae	*Abies*	*Abies pindrow*	Pakistan	Pakistan	Fever
570	0	Piperaceae	*Piper*	*Piper guineense* Schum & Thonn	Nigeria	Nigeria	Fever
571	0	Piperaceae	*Piper*	*Piper longum* L.	Nepal	Nepal	Fever
572	0	Piperaceae	*Piper*	*Piper sarmentosum* Roxb	Thailand	Thailand	Fever
573	0	Piperaceae	*Piper*	*Piper mullesua* Buch. Ham.	Northeast india	Northeast india	Malaria
574	0	Piperaceae	*Piper*	*Piper betle* L.	Indonesia	Indonesia	Malaria, Fever
575	AF275202.1	Piperaceae	*Piper*	*Piper spp.*	Multicultural	Indonesia, Thailand, Nigeria, Nepal, Northeast India	Malaria, Fever
576	0	Plantaginaceae	*Plantago*	*Plantago depressa* Willd	Nepal	Nepal	Fever
577	0	Plantaginaceae	*Plantago*	*Plantago psyllium* L.	Iran	Iran	Fever
578	AY101861.1	Plantaginaceae	*Plantago*	*Plantago spp*	Nepal	Nepal	Fever
579	KR259538.1	Plumbaginaceae	*Plumbago*	*Plumbago zeylanica* L.	Multicultural	Uganda, Limpopo, Meghalaya, India	Tuberculosis, Diarrhea
580	KR005618.1	Poaceae	*Coix*	*Coix lacryma-jobi*	Meghalaya	Meghalaya	Diarrhea
581	FJ410314.1	poaceae	*Bambusa*	*Bambusa vulgaris*	India	India	Fever
582	0	Poaceae	*saccharum*	*Saccharum officinarum*	Limpopo	Limpopo	Malaria, Fever, Tuberculosis, Diarrhea
583	MN781147.1	Poaceae	*Pennisetum*	*Pennisetum glaucum* L	Limpopo	Limpopo	Tuberculosis
584	MH762138.1	Poaceae	*Sorghum*	*Sorghum bicolor* (L.)	Limpopo	Limpopo	Tuberculosis
585	0	Podocarpaceae	*Podocarpus*	*Podocarpus usambarensis* Pilg	Uganda	Uganda	Tuberculosis
586	DQ372904.1	Polygalaceae	*Polygonum*	*Polygonum perfoliatum*	Meghalaya	Meghalaya	Diarrhea
587	0	Polygalaceae	*Polygala*	*Polygala persicariaefolia* DC.	Northeast india	Northeast india	Malaria
588	0	Polygonaceae	*Rheum*	*Rheum ribes*	Iran	Iran	Diarrhea
589	L78042.1	Portulacaceae	*Portulacaria*	*Portulacaria afra* Jag	Limpopo	Limpopo	Tuberculosis
590	0	Proteaceae	*Protea*	*Protea caffra* Meisn	Limpopo	Limpopo	Tuberculosis
591	DQ410718.1	Ranunculaceae	*ranunculus*	*Ranunculus muricatus*	Nigeria	Nigeria	Diarrhea
592	0	Ranunculaceae	*Aconitum*	*Aconitum heterophyllum* Wall. ex Royle	Nepal	Nepal	Fever
593	0	Ranunculaceae	*Aconitum*	*Aconitum spicatum* (Bruhl) Stapf	Nepal	Nepal	Fever
594	KM887365.1	Ranunculaceae	*Aconitum*	*Aconitum spp.*	Nepal	Nepal	Fever
595	0	Ranunculaceae	*Aconitum*	*Aconitum violaceum* Jacq. ex Stapf	Nepal	Nepal	Fever
596	KC815302.1	Ranunculaceae	*Coptis*	*Coptis teeta* Wall	Northeast india	Northeast india	Malaria
597	JX233693.1	Ranunculaceae	*Thalictrum*	*Thalictrum foliolosum* DC	Multicultural	Nepal, Northeast india	Malaria, Fever
598	0	Rhamnaceae	*Ziziphus*	*Ziziphus mauritiana*	Nepal	Nepal	Diarrhea
599	0	rhamnaceae	*Ziziphus*	*Ziziphus zeyheriana* Sond.	Limpopo	Limpopo	Tuberculosis
600	KR083102.1	Rhamnaceae	*Ziziphus*	*Ziziphus spp*	Multicultural	Limpopo, Bizana, Nepal	Tuberculosis, Diarrhea
601	0	Rosaceae	*Cerasus*	*Cerasus brachypetala*	Iran	Iran	Diarrhea
602	KF912900.1	Rosaceae	*Duchesnea*	*Duchesnea indica/ Potentilla indica*	Nigeria	Nigeria	Diarrhea
603	FJ449737.1	Rosaceae	*Eriobotrya*	*Eriobotrya japonica* (Thunb.)	Limpopo	Limpopo	Tuberculosis
604	EU669109.1	Rosaceae	*Prunus*	*Prunus africana* (Hook.f.) Kalkman	Multicultural	Uganda, Bizana	Tuberculosis, Diarrhea
605	MG995011.1	Rubiaceae	*Catunaregam*	*Catunaregam spinosa*	Nepal	Nepal	Diarrhea
606	MH432188.1	Rubiaceae	*Neolamarckia*	*Neolamarckia cadamba*	West bengal	West bengal	Diarrhea

607	KM592313.1	Rubiaceae	*Pavetta*	*Pavetta indica*	West bengal	West bengal	Diarrhea
608	AM267033.1	Rubiaceae	*Pentanisia*	*Pentanisia prunelloides*	Bizana	Bizana	Diarrhea
609	KF488111.1	Rubiaceae	*Vangueria*	*Vangueria infausta*	Maputaland	Maputaland	Diarrhea
610	0	Rubiaceae	*Gardenia*	*Gardenia gummifera*	India	India	Diarrhea
611	0	Rubiaceae	*Morinda*	*Morinda tinctoria*	India	India	Diarrhea
612	0	Rubiaceae	*Spermacoce*	*Spermacoce ocymoides*	India	India	Diarrhea
613	AJ492631.1	Rubiaceae	*Adina*	*Adina cordifolia Wild/Haldina cordifolia*	Nepal	Nepal	Fever
614	0	Rubiaceae	*Galium*	*Galium pauciflorum* Bunge	Nepal	Nepal	Fever
615	0	Rubiaceae	*Musenda*	*Musenda frondosa* L	Nepal	Nepal	Fever
616	MK607927.1	Rubiaceae	*Paedaria*	*Paederia foetida* L	Multicultural	Nepal, Meghalaya	Fever, Diarrhea
617	MH710785.1	Rubiaceae	*Rubia*	*Rubia cordifolia L*	Multicultural	Nepal, Uganda	Fever, Tuberculosis
618	0	Rubiaceae	*Aganthesanthemum*	*Aganthesanthemum bojeri* Klotzsch	Kenya	Kenya	Malaria
619	AJ617752.1	Rubiaceae	*Canthium*	*Canthium glaucum* Hiern.	Kenya	Kenya	Malaria
620	AY538354.1	Rubiaceae	*Cinchona*	*Cinchona officinalis* Linn f.	Northeast india	Northeast india	Malaria
621	MF178591.1	Rubiaceae	*Craterispermum*	*Craterispermum laurinum* (Poir.) Benth	Ivory coast	Ivory coast	Malaria
622	JX111223.1	Rubiaceae	*Hedyotis*	*Hedyotis scandens* Roxb.	Northeast india	Northeast india	Malaria
623	0	Rubiaceae	*Morinda*	*Morinda chrysorhiza* DC	Ivory coast	Ivory coast	Malaria
624	AJ346899.1	Rubiaceae	*Nauclea*	*Nauclea latifolia* Sm/*Sarcocephalus latifolius*	Multicultural	nigeria, Ivory coast, Ghana	Malaria
625	0	Rubiaceae	*Pentas*	*Pentas agathisanthemum* Kl.	Kenya	Kenya	Malaria
626	AM267049.1	Rubiaceae	*Pentas*	*Pentas longiflora* Oliv	Kenya	Kenya	Malaria
627	FJ907073.1	Rubiaceae	*Morinda*	*Morinda spp*	Multicultural	Nigeria, Ghana, Uganda, Ivory coast, India	Malaria, Fever, Tuberculosis, Diarrhea
628	AY845130.1	Rubiaceae	*Moringa*	*Moringa oleifera* Lam	Multicultural	Nigeria, Bangladesh, Uganda, Zimbabwe	Malaria, Fever, Tuberculosis, Diarrhea
629	DQ153593.1	Rubiaceae	*Coffea*	*Coffea canephora* Pierre ex A. Froehner	Uganda	Uganda	Tuberculosis
630	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum armatum*	Nepal	Nepal	Diarrhea
631	JX144214.1	Rutaceae	*Murraya*	*Murraya koenigii*	India	India	Diarrhea
632	0	Rutaceae	*Atalantia*	*Atalantia racemosa*	India	India	Fever
633	0	Rutaceae	*Citrus*	*Citrus limon* (L.) Burm.f	Nigeria	Nigeria	Fever
634	0	Rutaceae	*Evodia*	*Evodia fraxinifolia* Hook	Nepal	Nepal	Fever
635	KX277675.1	Rutaceae	*Skimmia*	*Skimmia anquetilia* N.P. Taylor & Airy Shaw	Nepal	Nepal	Fever
636	FJ434169.1	Rutaceae	*Aegle*	*Aegle marmelos* (L.)	Multicultural	Bangladesh, Meghalaya, Nepal	Fever, Diarrhea
637	0	Rutaceae	*Citrus*	*Citrus aurantium* L.	Nigeria	Nigeria	Malaria
638	0	Rutaceae	*Citrus*	*Citrus sinensis* (L.) Osbeck	Northeast india	Northeast india	Malaria
639	JX144189.1	Rutaceae	*Clausena*	*Clausena excavata* Burm. f	Northeast india	Northeast india	Malaria
640	0	Rutaceae	*Fagara*	*Fagara macrophylla* (Oliv.)	Ivory coast	Ivory coast	Malaria
641	KU193665.1	Rutaceae	*Fagaropsis*	*Fagaropsis angolensis* (Engl) Del	Kenya	Kenya	Malaria
642	KU193675.1	Rutaceae	*Teclea*	*Teclea simplicifolia* (Eng) Verdoon	Kenya	Kenya	Malaria
643	MN257824.1	Rutaceae	*Vepris*	*Vepris ampody* H. Perr.	Madagascar	Madagascar	Malaria
644	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum hamiltonianum* Wall.	Northeast india	Northeast india	Malaria
645	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum tsihanimposa* Bak	Madagascar	Madagascar	Malaria

646	0	Rutaceae	*Citrus*	*Citrus medica* L	Multicultural	Northeast india, Meghalaya	Malaria, Diarrhea
647	0	Rutaceae	*Citrus*	*Citrus paradisi* L.	Nigeria	Nigeria	Malaria, Fever
648	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum alatum* Roxb	Nepal	Nepal	Malaria, Fever
649	FJ641964.1	Rutaceae	*Citrus*	*Citrus spp.*	Multicultural	Nigeria, Ghana, Northeast india, Meghalaya	Malaria, Fever, Diarrhea
650	MH016555.1	Rutaceae	*Zanthoxylum*	*Zanthoxylum spp*	Multicultural	Nigeria, Ghana, Kenya, Limpopo, Nepal	Malaria, Fever, Tuberculosis, Diarrhea
651	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum chalybeum* Engl.	Multicultural	Kenya, Uganda	Malaria, Tuberculosis
652	KU193662.1	Rutaceae	*Citropsis*	*Citropsis articulata* (Willd. ex Spreng.) Swingle & M. Kellerm	Uganda	Uganda	Tuberculosis
653	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum humile* (E.A.Bruce)	Limpopo	Limpopo	Tuberculosis
654	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum leprieurii* Guill. & Perr	Uganda	Uganda	Tuberculosis
655	JF978978.1	Rutaceae	*Toddalia*	*Toddalia asiatica* (L.) Lam	Multicultural	Uganda, India	Tuberculosis, Diarrhea
656	0	Rutaceae	*Zanthoxylum*	*Zanthoxylum capense* (Thunb.)	Multicultural	Limpopo, Bizana	Tuberculosis, Diarrhea
657	MH711020.1	Salicaceae	*Salix*	*Salix babylonica* L	Nepal	Nepal	Fever
658	0	Salicaceae	*Trimeria*	*Trimeria grandifolia* (Hochst.) Warb.	Uganda	Uganda	Tuberculosis
659	KJ137254.1	santalaceae	*Santalum*	*Santalum album*	India	India	Fever
660	MT137488.1	Santalaceae	*Osyris*	*Osyris lanceolata* Hochst. &Steud.	Limpopo	Limpopo	Tuberculosis, Diarrhea
661	EU720424.1	Sapindaceae	*Pappea*	*Pappea capensis*	Limpopo	Limpopo	Diarrhea
662	0	Sapindaceae	*Sapindus*	*Sapindus emarginata*	India	India	Diarrhea
663	0	Sapindaceae	*Nephelium*	*Nephelium mutabile*	Malaysia	Malaysia	Fever
664	KX584899.1	Sapindaceae	*Allophylus*	*Allophylus pervillei* Blume.	Nigeria	Nigeria	Malaria
665	0	Sapindaceae	*Deinbollia*	*Deinbollia pinnata* Schum.& Thonn	Ghana	Ghana	Malaria
666	JN190997.1	Sapindaceae	*Lecaniodiscus*	*Lecaniodiscus cupanioides*	Nigeria	Nigeria	Malaria
667	KX584937.1	Sapindaceae	*Paullinia*	*Paullinia pinnata* L	Ghana	Ghana	Malaria
668	JN190976.1	Sapindaceae	*Blighia*	*Blighia unijugata* Baker	Uganda	Uganda	Tuberculosis
669	FJ546974.1	Sapindaceae	*Dodonaea*	*Dodonaea viscosa* Jacq.	Limpopo	Limpopo	Tuberculosis
670	KX545256.1	Sapotaceae	*Madhuca*	*Madhuca* sp	India	India	Diarrhea
671	0	Sapotaceae	*Mimusops*	*Mimusops elengi*	India	India	Diarrhea
672	KF686306.1	Sapotaceae	*Vitellaria*	*Vitellaria paradoxa* (Gaertn. f.)	Nigeria	Nigeria	Fever
673	0	Sapotaceae	*Chrysophyllum*	*Chrysophyllum albidum* G. Don	Nigeria	Nigeria	Malaria
674	KF686247.1	Sapotaceae	*Mimusops*	*Mimusops spp*	Multicultural	Limpopo, India	Tuberculosis, Diarrhea
675	JN102224.1	Saxifragaceae	*Astilbe*	*Astilbe rivularis*	Himalaya	himalaya	Diarrhea
676	KY986451.1	Saxifragaceae	*Bergenia*	*Bergenia ciliata*	Nepal	Nepal	Diarrhea
677	KX065314.1	Saxifragaceae	*Hydrangea*	*Hydrangea macrophylla* (Thunb.) Ser	Northeast india	Northeast india	Malaria
678	0	Scrophulariaceae	*Aptosimum*	*Aptosimum lugardiae* (N.E.Br.ex Hemsl. & Skan)	Limpopo	Limpopo	Tuberculosis

679	0	Scrophulariaceae	*Buddleja*	*Buddleja salvifolia* (L.) Lam	Limpopo	Limpopo	Tuberculosis
680	0	Simaroubaceae	*Quassia*	*Quassia africana*	Congo	Congo	Diarrhea
681	AY510155.1	Simaroubaceae	*Brucea*	*Brucea javanica* (Linn.) Merr	Northeast india	Northeast india	Malaria
682	MN257686.1	Simaroubaceae	*Harrisonia*	*Harrisonia abyssinica* Oliv.	Multicultural	Kenya , kenya	Malaria
683	0	Simaroubaceae	*Irvingia*	*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill	Ivory coast	Ivory coast	Malaria
684	KR532487.1	Simaroubaceae	*Picrasma*	*Picrasma javanica* Bl	Northeast india	Northeast india	Malaria
685	0	Solanaceae	*Solanum*	*Solanum nigrum*	India	India	Diarrhea
686	HQ705990.1	Solanaceae	*Capsicum*	*Capsicum frutescens*	Nigeria	Nigeria	Fever
687	MH566981.1	Solanaceae	*Nicotiana*	*Nicotiana tabacum* SW. Afr	Nigeria	Nigeria	Fever
688	0	Solanaceae	*Solanum*	*Solanum torvum* Sw	Northeast india	Northeast india	Malaria
689	0	Solanaceae	*Solanum*	*Solanum vairum* Cl	Northeast india	Northeast india	Malaria
690	MH768322.1	Solanaceae	*Datura*	*Datura metel* L.	Multicultural	Northeast india, Pakistan	Malaria, Diarrhea
691	AY665875.1	Solanaceae	*Physalis*	*Physalis spp*	Multicultural	Nigeria, Bizana	Malaria, Diarrhea
692	KR425501.1	Solanaceae	*Solanum*	*Solanum spp.*	Multicultural	Nigeria, Northeast india, Limpopo, India	Malaria, Fever, Diarrhea
693	KX277729.1	Sterculiaceae	*Helicteres*	*Helicteres isora*	India	India	Diarrhea
694	MK696138.1	Steruliaceae	*Dombeya*	*Dombeya rotundifolia*	Limpopo	Limpopo	Diarrhea
695	AY074729.1	Steruliaceae	*Theombroma*	*Theobroma cacao* L	Ghana	Ghana	Malaria
696	0	Steruliaceae	*Sterculia*	*Sterculia setigera* Delile.	Senegal	Senegal	Tuberculosis
697	0	Sympolaceae	*Sympolocos*	*Sympolocos racemosa*	Meghalaya	Meghalaya	Diarrhea
698	AJ744928.1	Thymelaeaceae	*Dais*	*Dais cotinifolia*	Bizana	Bizana	Diarrhea
699	AJ744920.1	Thymelaeaceae	*Peddiea*	*Peddiea involucrata* Bak.	Madagascar	Madagascar	Malaria
700	0	Thymelaeaceae	*Lasiosiphon*	*Lasiosiphon caffer* Meisn	Limpopo	Limpopo	Tuberculosis
701	0	Tiliaceae	Grewia	*Grewia hexaminta* Burret.	Kenya	Kenya	Malaria
702	0	Tiliaceae	*Grewia*	*Grewia plagiophylla* K. Schum	Kenya	Kenya	Malaria, Fever
703	0	ulmaceae	*Trema*	*Trema spp*	Multicultural	Nigeria, Bizana	Fever, Diarrhea
704	KC539583.1	Ulmaceae	*Chaetacme*	*Chaetacme aristata* Planch.	Uganda	Uganda	Tuberculosis
705	HQ377205.1	umbeliferae	*Coriandrum*	*Coriandrum sativum*	India	India	Fever
706	KF137835.1	Urticaceae	*Debregeasia*	*Debregeasia saeneb*	Pakistan	Pakistan	Fever
707	0	urticaceae	*Fleurya*	*Fleurya aestuans* (L.) Gaudich. ex Miq	Uganda	Uganda	Tuberculosis
708	KF137916.1	Urticaceae	*Pouzolzia*	*Pouzolzia mixta* Solms var.mixta	Limpopo	Limpopo	Tuberculosis
709	0	Valerianaceae	*Nardostachys*	*Nardostachys grandiflora*	Nepal	Nepal	Diarrhea
710	KX277663.1	Valerianaceae	*Valeriana*	*Valeriana jatamansi*	Nepal	Nepal	Diarrhea
711	AY236190.1	Valerianaceae	*Nardostachys*	*Nardostachys spp*	Nepal	Nepal	Fever, Diarrhea
712	JN016988.1	Velloziaceae	*Xerophyta*	*Xerophyta retinervis* Bake	Limpopo	Limpopo	Tuberculosis
713	MH711742.1	Verbenaceae	Vitex	*Vitex negundo* L	Multicultural	Nepal, Bangladesh, India	Fever, Diarrhea
714	KT728416.1	Verbenaceae	*Clerodendrum*	*Clerodendrum spp*	Northeast india	Northeast india	Malaria, Tuberculosis
715	0	Verbenaceae	*Clerodendrum*	*Clerodendrum colebrookoianum* Walp	Northeast india	Northeast india	Malaria

716	0	Verbenaceae	*Clerodendrum*	*Clerodendrum serratum* (L.) Moon/*Rotheca serrata*	Northeast india	Northeast india	Malaria
717	0	Verbenaceae	*Premna*	*Premna chrysoclada* (Boger) Gürke	Kenya	Kenya	Malaria
718	0	Verbenaceae	*Premna*	*Premna sp. (cf. glandulosa* Merr.)	Indonesia	Indonesia	Malaria
719	0	Verbenaceae	*Vitex*	*Vitex peduncularis* Wall	Northeast india	Northeast india	Malaria
720	MH768342.1	Verbenaceae	*Lantana*	*Lantana camara* L.	Multicultural	Northeast india, Kenya, Uganda, Limpopo, India	Malaria, Tuberculosis, Diarrhea
721	0	Verbenaceae	*Lantana*	*Lantana rugosa* Thunb	Limpopo	Limpopo	Tuberculosis
722	0	Verbenaceae	*Lippia*	*Lippia chevalieri* Moldenkes	Senegal	Senegal	Tuberculosis
723	0	Verbenaceae	*Lippia*	*Lippia grandifolia* Hochst. ex A. Rich	Uganda	Uganda	Tuberculosis
724	MK261289.1	Verbenaceae	*Lippia*	*Lippia javanica* (Burm f.) Spreng.	Multicultural	Uganda, Limpopo	Tuberculosis
725	0	Violaceae	*Viola*	*Viola canescens*	Pakistan	Pakistan	Fever
726	KT344571.1	Vitaceae	*Ampelocissus*	*Ampelocissus africana*	Zimbabwe	Zimbabwe	Diarrhea
727	0	Vitaceae	*Ampelocissus*	*Ampelocissus obtusata*	Zimbabwe	Zimbabwe	Diarrhea
728	0	Vitaceae	*Cissus*	*Cissus rubiginosa*	Congo	Congo	Diarrhea
729	0	Vitaceae	*Cyphostemma*	*Cyphostemma humile* (N.E.Br.)Desc. ex Wild & R.B.Drumm	Limpopo	Limpopo	Tuberculosis
730	0	Vitaceae	*Cyphostemma*	*Cyphostemma woodii* (Gilg &M.Brandt) Desc.	Limpopo	Limpopo	Tuberculosis
731	KT344623.1	Vitaceae	*Rhoicissus*	*Rhoicissus tridentata* (L.f.)Wild & R.B.Drumm	Multicultural	Limpopo, Bizana	Tuberculosis, Diarrhea
732	AF478715.1	Zingiberaceae	*Alpinia*	*Alpinia galanga*	India	India	Diarrhea
733	AF478792.1	Zingiberaceae	*Siphonochilus*	*Siphonochilus aethiopicus* (Schweinf.) B.L.Burtt	Limpopo	Limpopo	Tuberculosis

APPENDIX III

Phytochemical Screening Result (Picture)

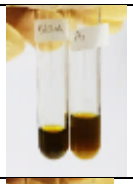
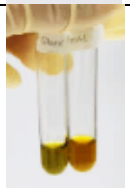
Table preliminary phytochemical screening result for alkaloids

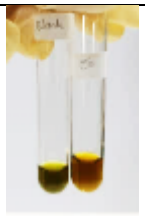
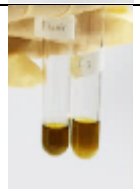
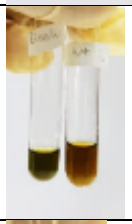
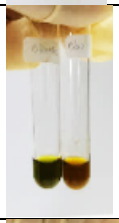
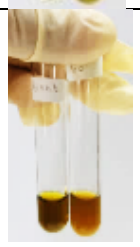
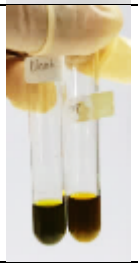
No	Species list	Source of collection	Dragendorff's test			Wagner's test		
			Result	Picture	Color of precipitate	Result	Picture	Color of precipitate
1	*A. vulgaris*	Tak Province	-		-	+		Brown
2	*A. lactiflora*	Chatuchak	+		Yellow white	+		Blackish
3	*A. dracunculus*	Nakhin Pathom	-		-	+		Brown
4	*A. chinensis*	Northaburi	-		-	-		-
5	*A. conyzoides*	Kumpangsan	+		Yellow white	+		Brown
6	*E. odoratum*	North Thailand	+		Orange red	+		Yellow black
7	*V. cinerea*	Northern Thai	+		Orange red	+		Black

8	*W. trilobata*	Bangkok	-		-	+		Red black
9	*T. procumbens*	Chiang Mai	+		Yellow orange	+		Yellow
10	*B. balsamifera*	North Thailand	+		Brown red	+		Yellow
11	*G. divaricata*	Chatuchak	-		-	+		Yellow black
12	*G. pseudochina*	Chatuchak	+		Yellow brown	+		Yellow brown
13	*B. pilosa*	Chiang Mai	+		Yellow orange	+		Yellow
14	*E. capilifolium*	Northaburi	+		Yellow orange	+		Black
15	*S. indicus*	Mukdahan	+		Yellow brown	+		Red Black

16	*A. oleracea*	Nakhon Sithumarat, South Thai	+		Orange	+		Yellow

Table preliminary phytochemical screening result of flavonoid (alkaline test)

No	Species list	Source	Alkaline test (flavonoid)		
			Result	Picture after addition of 5% NaOH	Picture after addition of 2M HCl
1	*A. vulgaris*	Tak Province	+		
2	*A. lactiflora*	Chatuchak	+		
3	*A. dracunculus*	Nakhin Pathom	+		
4	*A. chinensis*	Northaburi	++		
5	*A. conyzoides*	Kumpangsan	+-		

6	*E. odoratum*	North Thailand	+		
7	*C. cinereum*	Northern Thai	+		
8	*W. trilobata*	Bangkok	+		
9	*T. procumbens*	Chiang Mai	+		
10	*B. balsamifera*	North Thailand	+		
11	*G. divaricata*	Chatuchak	+-		
12	*G. pseudochina*	Chatuchak	+		

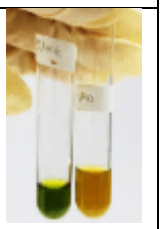
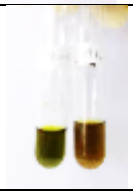
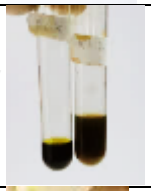
13	*B. pilosa*	Chiang Mai	++		
14	*E. capilifolium*	Northaburi	+		
15	*S. indicus*	Mukdahan	++		
16	*A. oleracea*	Nakhon Sithumarat, South Thai	+		

Table preliminary phytochemical screening result of flavonoid (ferric chloride test)

No	Species list	Source		
1	*A. vulgaris*	Tak Province	++	
2	*A. lactiflora*	Chatuchak	+	

3	*A. dracunculus*	Nakhin Pathom	++	
4	*A. chinensis*	Northaburi	++	
5	*A. conyzoides*	Kumpangsan	+	
6	*E. odoratum*	North Thailand	++	
7	*C. cinereum*	Northern Thai	++	
8	*W. trilobata*	Bangkok	++	
9	*T. procumbens*	Chiang Mai	+	
10	*B. balsamifera*	North Thailand	++	

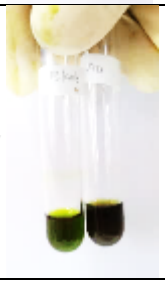
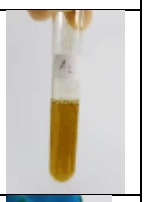
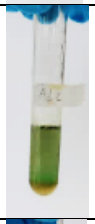
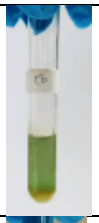
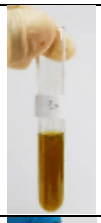
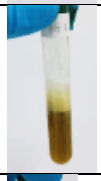
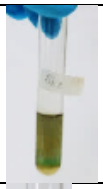
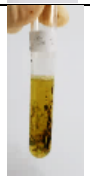
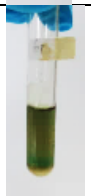
11	*G. divaricata*	Chatuchak	++	-
12	*G. pseudochina*	Chatuchak	++	
13	*B. pilosa*	Chiang Mai	++	
14	*E. capilifolium*	Northaburi	+	
15	*S. indicus*	Mukdahan	++	
16	*A. oleracea*	Nakhon Sithumarat, South Thai	++	

Table preliminary phytochemical screening result of triterpenes and steroids (salkowski test)

No	Species list	Source	Triterpenes	Steroids	Picture	Saponin	
			Result	Result		Result	Picture
1	*A. vulgaris*	Tak Province	+	+		-	
2	*A. lactiflora*	Chatuchak	+	+		-	
3	*A. dracunculus*	Nakhin Pathom	+	-		-	
4	*A. chinensis*	Northaburi	+	+		-	
5	*A. conyzoides*	Kumpangsan	+	+		+	

6	*E. odoratum*	North Thailand	+	+		-	
7	*C. cinereum*	Northern Thai	+	+		-	
8	*W. trilobata*	Bangkok	+	+		+	
9	*T. procumbens*	Chiang Mai	+	+		-	
10	*B. balsamifera*	North Thailand	+	+		+-	
11	*G. divaricata*	Chatuchak	-	+		+	
12	*G. pseudochina*	Chatuchak	-	-		-	
13	*B. pilosa*	Chiang Mai	+	+		+	
14	*E. capilifolium*	Northaburi	+	+		-	

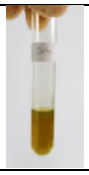
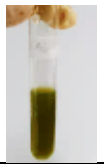
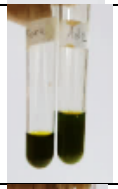
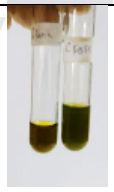
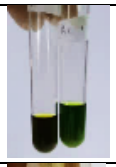
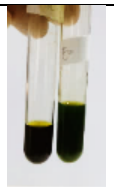
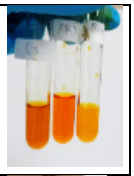
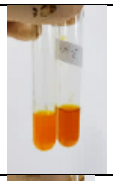
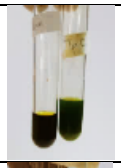
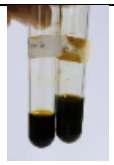
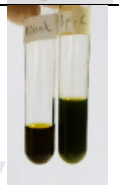
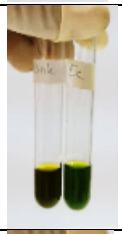
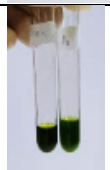
15	*S. indicus*	Mukdahan	+	-		-	
16	*A. oleracea*	Nakhon Sithumarat, South Thai	+	+		-	

Table preliminary phytochemical screening result for diterpenes and lactones

No	Species list	Source	diterpenes	Result	lactones	Result
1	*A. vulgaris*	Tak Province	+		+	
2	*A. lactiflora*	Chatuchak	+		+	
3	*A. dracunculus*	Nakhin Pathom	+-		+	
4	*A. chinensis*	Northaburi	+-		+	
5	*A. conyzoides*	Kumpangsan	+		+	
6	*E. odoratum*	North Thailand	+		+	

7	*C. cinereum*	Northern Thai	+		+	
8	*W. trilobata*	Bangkok	+		+	
9	*T. procumbens*	Chiang Mai	+		+	
10	*B. balsamifera*	North Thailand	+		+	
11	*G. divaricata*	Chatuchak	-		-	
12	*G. pseudochina*	Chatuchak	+		-	
13	*B. pilosa*	Chiang Mai	+-		+	
14	*E. capilifolium*	Northaburi	+		+	
15	*S. indicus*	Mukdahan	+-		+	
16	*A. oleracea*	Nakhon Sithumarat, South Thai	+		-	

APPENDIX IV

Phytochemical Screening Reagents Preparation

1. Preparation of Dragendorff's reagent

Reagent	: Bismuth (III) subnitrate, SIGMA ALDRICH
	KI (Potassium iodide), AnalaR, BDH Chemicals Ltd
	Glacial acetic acid

Procedure:

Preparation of 10 mL stock solution

- **Step 1**: making 50 % potassium iodide by dissolving 2,5 g of KI into H_2O until obtained 5 mL of solution.
- **Step 2:** 85 mg of bismuth (III) subnitrate is dissolved into 4 mL of H_2O and stir, then followed by adding 1 mL of glacial acetic acid. After stirring, add 5 mL of 50% potassium iodide solution and stir until dissolved completely. Keep in the dark bottle.

Preparation of 100 mL working solution

10 mL of stock solution is added with 20 mL of glacial acetic acid, then add the solution into 70 ml of H_2O. Keep the working solution in the dark bottle.

2. Preparation of Wagner's reagent

Reagent	: Iodine,
	KI (Potassium iodide), AnalaR, BDH Chemicals Ltd
	Glacial acetic acid

Procedure:

Dissolve 2 g of iodine and 6 g of KI into 100 mL of H_2O.

3. Preparation of 1% diluted acid

Reagent: conc. HCl 37%,

Procedure:

27,027 mL of conc. HCl (37%) is added into 972,973 mL of H_2O.

4. Preparation of 25 mL 5% Ferric Chloride reagent

Reagent: FeCl3.6H_2O, Iron (III) chloride hexahydrate Pure P.A., POCH SA

Procedure:

Dissolve 1,25 g of $FeCl_3$.6H_2O into 25 ml of H_2O.

5. Preparation of 50 mL 5% NaOH

Procedure:

Dissolve 2,5 gram of NaOH into 50 mL of H_2O

6. Preparation of 50 mL 10% NaOH

Procedure:

Dissolve 5 gram of NaOH into 50 mL of H_2O

7. Preparation of 40 mL 2M HCl

37 % HCl equivalent with 12 M HCl

Procedure:

Add 6,66 ml of conc. HCl into 33.34 mL of H_2O.

8. Preparation of 1000 mL 1 % diluted HCl

1 %.1000 mL = 37%.X

X = 27,027 mL

Procedure:

Add 27,027 ml conc. HCl into 972.973 ml H_2O.

9. Preparation of 1% Copper acetate reagent

Reagent: Copper acetate,

Procedure:

Dissolve 1 g copper acetate into 100 ml of H_2O.

10. Preparation of Baljet reagent

Reagent	: saturated Picric acid (1,3 % in water)
	NaOH 10 %
	Ethanol

Procedure:

Prepare freshly before using the reagent. Add the saturated picric acid into 10% NaOH with ratio 1:1

APPENDIX V

Preparation of 2XCTAB Buffer

Composition of CTAB Buffer:

Stock	**Final Concentration**	**Amount**
CTAB	2% (W/V)	2 g
1 M Tris-HCl pH 8	100 mM	10 ml
0,5 EDTA pH 8	20 mM	4 ml
5 M NaCl	1.4 M	28 ml
PVP	1%	1 g

These components were made up to 100 mL in water. And added 4 µL of 2-mercaptoethanol to each 1 mL of 2XCTAB buffer before used.

VITA

NAME	Desy Liana
DATE OF BIRTH	13 October 1993
PLACE OF BIRTH	Bantul
INSTITUTIONS ATTENDED	Faculty of Biology, Gadjah Mada University, Indonesia
HOME ADDRESS	Potrobayan RT002, Srihardono, Pundong, Bantul, Yogyakarta Indonesia, 55771

VITA

NAME	Desy Liana
DATE OF BIRTH	13 October 1993
PLACE OF BIRTH	Bantul
INSTITUTIONS ATTENDED	Faculty of Biology, Gadjah Mada University, Indonesia
HOME ADDRESS	Potrobayan RT002, Srihardono, Pundong, Bantul, Yogyakarta Indonesia, 55771