

Pharmacognostic Study on the Raw Materials Present In Some Medicinal Plants and Their Antimalarial Effect

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Abstract

The selected plants *Samanea saman* (Jacq.) Merr. (Kalar-kokko) belongs to the family Mimosaceae; *Dactyloctenium aegyptium* (L.) Willd. (Myet-thone-kha) belongs to the family Poaceae; *Brucea javanica* (L.) Merr. (Yadan-zi) belongs to the family Simaroubaceae; *Plumeria alba* L. (Tayok-saga-aphyu) belongs to the family Apocynaceae and *Ferula foetida* Regal. (Shein-go) belongs to the family Apiaceae. These plants are widely distributed in Myanmar. In morphological, sensory characters, macroscopical and diagnostic characters study of some medicinal plants of powdered simple and pitted vessels, fibres, fibre-tracheids, tracheids, laticiferous cells, oil cells and the fragments of epidermal cells. In physicochemical properties of some medicinal plants, methanol extracts showed more soluble than other solvents. These some medicinal plants are locally well-known as especially antimalarial and also employed for the treatment of several cancer and various diseases. One of the potential sources of antimalarial drugs is traditional medicinal plants. This study is to determine the *in vitro* antimalarial effect of methanolic extracts of combination of some medicinal plants on *Plasmodium falciparum*. Twenty blood samples from *Plasmodium falciparum* infected patients which fulfilled the selection criteria were investigated for *in vitro* drug sensitivity test (WHO, 1987, 2001). Test doses of methanolic extracts of some medicinal plants were used ranging from (6.25-400 µg/ml). Methanolic extracts of combination of some medicinal plants showed significant antimalarial effect (EC₅₀ values = 19.44 µg/ml). Therefore this methanolic extracts of combination of some medicinal plants showed highly active antimalarial effect on *Plasmodium falciparum*.

Keywords: antimalarial effect, physicochemical properties

I. Introduction

In Myanmar, the Government has put enormous effort for the development of traditional medicines. Safe and effective traditional medicine have been identified and sought for the treatment of six major selected diseases such as malaria, diabetes, hypertension, dysentery, diarrhoea and tuberculosis. Rational use and further development of these herbal medicines, medicinal plants will be supported by further scientific studies.

The first antimalarial drug from plant origin was quinine from the bark of cinchona. Even today quinine remains an important and effective treatment for malaria in most parts of the world, although resistance has been reported in 1844 and 1910 (Kakkilaya, 2006).

The search for other antimalarial drugs from natural sources has also continued. In 1940, another antimalarial drug chloroquine was synthesized and it becomes a magic bullet to kill all species of malaria parasites until 1959-1960. Unfortunately, after an early success, the malarial parasite especially *Plasmodium falciparum* became resistant to chloroquine (Neerija, 2003).

Later a plant-based antimalarial drug has been isolated from the Chinese plant *Artemisia annua*, in the late 1970s and it is nominated as artemisinin which isolated from

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Artemisia annua an herb traditionally used in Chinese medicine for treating intestinal parasites, and febrile diseases, has been proved to be the most effective treatment against Malaria. Artemisinin in other parts of the world has been used in treatment of *Plasmodium falciparum* malaria cases around the world (Carvalho, 1991).

Bruceantin from *Brucea javanica* is also active chloroquine resistant strains of *Plasmodium falciparum*. In an effort to obtain a better safe wide-spectrum chemotherapeutic antimalarial agent (Purnima, 1992).

Malaria is a public health problem in more than 90 countries inhabited by 2.4 billion people. It is responsible for > 500 million clinical cases and 1.5-2.7 million deaths per year, most of whom are children under 5 years of age and pregnant women (Schwartlander, 1997 and WHO, 1996).

Current anti-protozoal drugs are inadequate due to parasite resistance, toxicity, lack of efficacy and inability to eliminate all stages of parasites from the host (Tasdemir, 2005).

However with the increase in cases of drug resistance and failure, there is an increase in the use of herbal medicine for the management of disease conditions (Phillipson, 1991).

Myanmar is rich in varieties of medicinal as well as aromatic plants due to the presence of different climate zones in the country. There are 7000 different known plants growing in Myanmar and most of them have been recognized as medicinal plants. Plant derived medicines have been utilized for antimalarial activity due to the traditional therapeutics uses for many years in Myanmar (Hundley and Chit Ko Ko, 1961).

Samanea saman (Jacq.) Merr. is indigenous to Northern part of South America. It is widely distributed in most tropical countries (Hutchison, 1964). The tree *Samanea saman* (Jacq.) Merr. is commonly known as "Rain Tree" and it is traditionally called "Thinbawkokko" or "Kaka-kokko". It is also cultivated as an ornamental shade tree. The family Mimosaceae consists of 56 genera and about 2800 species (The Wealth of India, 1952).

Dactyloctenium aegyptium (L.) Willd. is common throughout the plants of India. The grass growing in New South Wales is reported to contain cyanogenetics glycosides. In Indian medicine, the grass is used for imparting medicinal properties of Takra (buttermilk) in intestinal, biliary and urinary diseases, astringent, bitter tonic, anthelmintic, polyurea, externally for wounds and ulcers (Khare, 2007).

Brucea javanica (L.) Merr. Synonyms: *Brucea sumatrana* Roxb., *Brucea amarissima* (Lour.) Desv. Ex Gomes. *Brucea javanica* (L.) Merr. is widespread and occurs from Sri Lanka and India towards Indo-china, Southern China, Taiwan, Thailand, and although rare in the Molucous and New Guinen throughout the Malesian region to northern Australia (Padua, 1999); Grows wild, common on the sea-coast. *Brucea javanica* used for most often the pyrenes and roots, are used medicinally, mainly against amoebic dysentery, diarrhoea, malaria and as a febrifuge. It is known in Chinese traditional medicine, where it is additionally applied for the treatment of haemorrhoids, corns, warts ulcers and cancer. The pyrenes are well-known under the name 'Macassar kernels', and are also applied as an insecticide. The leaves are applied as a poultice against enlarged spleen, scurf, ring-worm, boils and centipede bites. A decoction of the roots is also used to treat abdominal pains, coughs and as an important remedy for internal poisoning. In Australia, the bark and roots have been used by Aborigines to treat toothache. The seeds are used as a parasiticide. They are effective for amoebiasis. They are active in malaria. The oil is extracted from the seeds to

ovoid its vomitive effect. Recta injection of an aqueous maceration is less toxic. A poultice of pounded seeds relieves haemorrhoids (Prajapati, 2003).

Plumeria alba L. also called "Pagoda Tree", or "Temple Tree", is a genus of laticiferous trees and shrubs, native of tropical America. It comprises of about fifty species. These plants are used for malaria, leprosy, rheumatism and abdominal tumors. The fruits of *Plumeria alba* L. is also edible. The latex of stem and leaves is applied to purgative, cardiotoxic, diuretic, hypotensive, ulcer, herpes and scabies and the seeds are said to possess haemostatic properties. The roots are cathartic (The Wealth of India, 1969).

Ferula, "hing" is a genus of one hundreds and thirty species and four are native to India. The species is native to Afghanistan, Pakistan, Iran, Kashmir and Tibet. It is dried latex exuded from the leaves. Hing is acrid and bitter in taste and emits a pungent smell (Indian council of Medical Research, 1987 and Padua, 1999).

The more important ferula gum-resins imported into India chiefly from Persia and Afghanistan are asafoetida, galbanum and sumbol. A part of the imported resins is re-exported to various countries. The main regions of production of asafetida are eastern Persia and western Afghanistan whence the drug is exported via Persian imports to Bombay. Asafoetida is acrid and bitter in taste and emits a strong alliaceous odour. It is extensively used in India for flavouring curries, sauces and pickles. Medicinally, it stimulates the intestinal and respiratory tracts and the nervous system. It is useful in asthma, whooping cough, and chronic bronchitis, an enema for intestinal flatulence, hysterical, epileptic affections, veterinary medicine and in cholera (The Wealth of India, 1956).

Asafoetida used for stimulant, carminative, antispasmodic, expectorant and mild laxative. It is also anthelmintic, diuretic, aphrodisiac and emmenagogue. It is a nervine and pulmonary stimulant. It acts on the organs of circulation and secretion which it stimulates (Indian council of Medical Research, 1987; Padua, 1999).

It is used for stimulant, carminative, antispasmodic, expectorant, and slightly laxative, anthelmintic, diuretic, aphrodisiac, emmenagogue, nervine, pulmonary stimulant and also increases the sexual appetite. Asafoetida produced slight inhibitions of growth of *Staphylococcus aureus* and *Shigella sonnei* (Kapoor, 2000).

According to the local people, it is very useful as the traditional folk medicine. Therefore, the aim of the present research is the development of plants in Myanmar traditional medicine based on scientific validation of its therapeutic effects and to promote an intensive application of it in Myanmar traditional medicine.

The selected plants *Samanea saman* (Jacq.) Merr. (Leaves), *Dactyloctenium aegyptium* (L.) Willd. (The whole plant), *Brucea javanica* (L.) Merr. (Seeds), *Plumeria alba* L. (Roots) and *Ferula foetida* Regal. (Gum-resin) are especially employed for the treatment of antimalarial in Myanmar (TMF-58). The results of this research will make considerable contribution to use in clinical research on malarial patients.

This research has been carried out with the objectives of identifying of *Samanea saman* (Jacq.) Merr., *Dactyloctenium aegyptium* L., *Brucea javanica* (L.) Merr., *Plumeria alba* L. and *Ferula foetida* Regal.; determination the sensory characters, macroscopical and diagnostic characters of the powdered of five medicinal plants; the physicochemical properties of five medicinal plants powdered and investigation of the *in vitro* antimalarial effect of methanolic extracts of combination of five medicinal plants on *Plasmodium falciparum*.

II. Materials and Methods

Collection and identification of five medicinal plants

The specimens used in this research were collected during flowering and fruiting periods 2013-2015. The specimens of *Samanea saman* (Jacq.) Merr., *Dactyloctenium aegyptium* L., *Plumeria alba* L. were collected from University of Yangon Campus; *Brucea javanica* (L.) Merr., was collected from Myeik in Tanintharyi Region and *Ferula foetida* Regal. was collected and identification from Nay Pyi Taw. The morphological characters were identified with the literatures of (Bailey, 1961; Backer, 1963; Hutchison, 1964; Hooker, 1885 and Prajapati, 2003). Diagnostic characters of specimen were examined by clearing reagent, chloral hydrate solution.

Extraction of five medicinal plants

The five samples were washed and cut into small pieces to dry faster. And then, both air-dried samples were pulverized and stored in air-tight containers to prevent from moisture and contamination. One hundred gram of combined samples the same ratio were extracted with methanol by using Rotatory evaporator.

Physicochemical properties of the combination of five medicinal plants powdered

The physicochemical properties of the combination of five medicinal plants powdered were determined according to "The British Pharmacopoeia" 1968 as follows:

Moisture content

The moisture contents of the combination of samples were determined by using an oven. Hundred gm of the dried sample was weighed and placed in a petridish and kept in an oven at 110°C for one hour. The petridish was then removed from the oven and cooled in a desiccator at room temperature and weighed. The procedure was repeated until a constant weight was obtained. The difference between the initial weight and final constant weight represented the moisture content of the sample. Then the percentage of the moisture content was calculated.

Total ash content

The combination of five medicinal plants powdered (100g) was replaced in a crucible and placed in a muffle-furnace at 475°C for 4 hrs until the substance turned into ash. The crucible was then cooled and weighed. The procedure was repeated until a constant weight was obtained and the percentage of total ash was calculated. The ash collected was divided into two to determine acid insoluble ash and water soluble ash.

Acid-insoluble ash

Half of the above ash was boiled for 5 min in 10ml of 6% dilute hydrochloric acid, and the insoluble matter was collected by vacuum filtration in a weighed crucible. The insoluble-ash was washed with boiling water until free of acid. The acid-insoluble residue together with the weighed crucible was dried in an oven at 105°C and weighed again. The percentage of the acid-insoluble ash was calculated.

Water-soluble ash

The other half of the total ash was boiled with 10 ml of water for 5 mins. The insoluble matter was collected on an ashless filter paper (Whatman paper no.42), washed with water and ignited to a constant weight. This weight of insoluble matter was subtracted from the weight of the total ash, to give weight of the water-soluble ash.

Petroleum ether soluble matter

Fifty gm of air-dried the combination of samples were soaked with 300 ml of pet-ether in a closed flask for 72 hrs and kept over three nights. The mixture was filtered rapidly taking precautions against loss of alcohol and the filtrate was evaporated in a weighed petridish on a boiling water bath, until it was completely dried. The evaporated residue together with the petridish was weighed. The procedure was repeated until a constant weight was obtained. The difference in weights gave the pet-ether soluble extractive value. The above procedure repeated with 30 ml of chloroform, ethyl acetate, acetone, methanol, ethanol, distilled water soluble matter instead of pet-ether. Then the respective soluble extractive values were calculated.

In Vitro* antimalarial effect of methanolic extracts of combination of five medicinal plants on *Plasmodium falciparum

In Vitro antimalarial effect were examined in Parasitology Research Division of Department of Medical Research (Lower).

Preparation of culture media

A synthetic medium RPMI 1640 (Grand Island Biological company, N.Y) was used for *in vitro* cultivation of *Plasmodium falciparum*. RPMI (1640) 10.4 g was dissolved in 900 ml of distilled water in volumetric flask. Then 5.94g of HEPES buffer, 0.01g of heparin and 2.0g of sodium bicarbonate were added and the total volume was adjusted to 1000ml with distilled water. Finally, the mixture was made until homogeneous solution by magnetic stirrer. The solution was filtered through 0.22 μ Millipore filter for sterilization and stored in 100ml screw capped bottle at 4°C until use.

Screening of subjects and ethical consideration

Patients who came to outpatient unit of malaria clinic, Shwe-kyin Township Hospital, were included in screening of subjects. Patients whose blood had to be taken for *in vitro* sensitivity assay were explained about the research. All subjects were questioned with respect to their recent history of taking antimalarial drugs. Persons who have received artemesinine or artemesinine derivatives within the last 7 days were excluded from the test. A patient, who had mono-infection with *Plasmodium falciparum* and asexual parasitaemia in excess of 2500 parasites, but less than 10, 0000 parasited per μ l blood, was considered suitable for testing. To exclude the effect of other drugs it made sure that parasites were healthy in appearance with appropriate number of rings and trophozoites.

All patients were treated with the appropriate drug immediately after taking blood samples for the test. All blood samples were used only for research purpose.

Collection of blood samples

The *Plasmodium falciparum* infected blood samples suitable for *in vitro* testing were selected after screening the patients. At least twenty blood samples which fulfilled the selection criteria were collected and subjected to the *in vitro* test system within 12hrs after

collection. The venous blood, 0.5ml was collected in each 1ml syringe and poured into a 5ml sterile screw-capped flacon tube containing 4.5ml RPMI medium.

Preparation of the test extracts

Test extracts, 0.01 g was dissolved in 1ml of EtOH. These solutions were filtered through Millipore sterile filters (Mesh 0.22 μm , Millipore SA, 67120, Molsheim, France) and then serial dilution were made with RPMI culture media solution to obtain required concentrations of 6.25 μg , 12.5 μg , 25 μg , 50 μg , 100 μg , 200 μg , 400 μg /ml solution.

Culture procedure and drug sensitivity assay

Drug sensitivity testing was performed following the micro technique using precharged plates (Rieckmann, 1978). Flat bottom microlitre culture plates with 8 x 12 wells were pre-dosed with increasing concentration (6.25, 12.5, 25, 50, 100, 200, 400 $\mu\text{g}/\text{ml}$) of respective extracts. Row (A) well of the plates filled with patient's culturing blood were kept blank to be used as control without drug. Dosing of test extracts were done starting from the well (B) and then following an increasing order of concentration of extracts up to well (H). All the wells of the each column were filled with 50 μl of blood: medium (1:9) using the appendorf pipette with a disposable sterile tip. A new disposable sterile tip was changed for every new row and the same way of blood: medium addition into each well was made. The microlitre plate was covered with lids and was shaken gently so that the drug deposits in the well were completely dissolved. The test plates were placed in a candle jar. When the flame became blue the jar was covered and incubated at 37°C for 30 hours.

After incubation, the supernatant in test wells were removed and the red blood cells deposited on the flat bottom of the wells were transferred onto a clean microscope slide to form a series of thick films. The resultant thick films were air dried for 24-48 hours. Then the thick films were stained for 45 minutes in a Giemsa stain at a dilution of 2% (v/v) in buffer water of P^H 6.8. Parasite count for each blood film was made using a microscope under oil immersion with X 100 objective.

Assessment of drug sensitivity

The assessment was made by counting the number of schizonts with 3 or more nuclei out of a total of 200 parasitized RBCs (schizonts and trophozoites) in each film. The number of schizonts in the control wells was taken as 100% base line for the assessment of schizonts maturation in the various drug wells. Growth inhibition was expressed as percent of the number of schizonts for each concentration, compared with untreated controls. Blood samples with the schizonts maturation of less than 10% in the control wells were not used for evaluation.

Data analysis

The antimalarial activity of this tested drug was expressed by effective concentration (EC₅₀) of the drug that induced 50% reduction in parasitaemia compared to the control (100% parasitaemia). The EC₅₀ value was calculated using (Wernsdorfer, 1995) software.

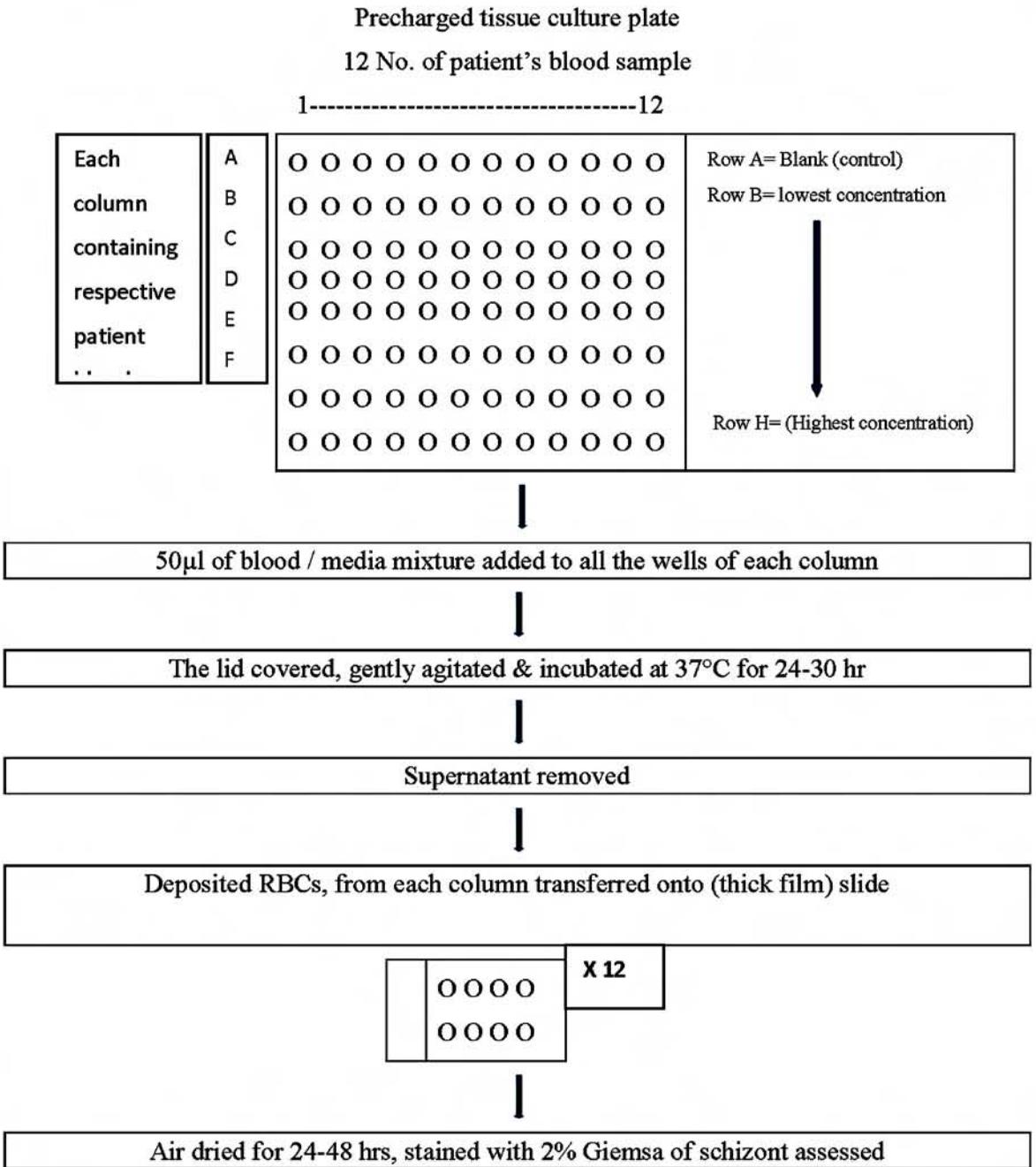


Figure 1. Flow chart of *in vitro* sensitivity assay of methanolic extracts of combination of five medicinal plants against *Plasmodium falciparum*

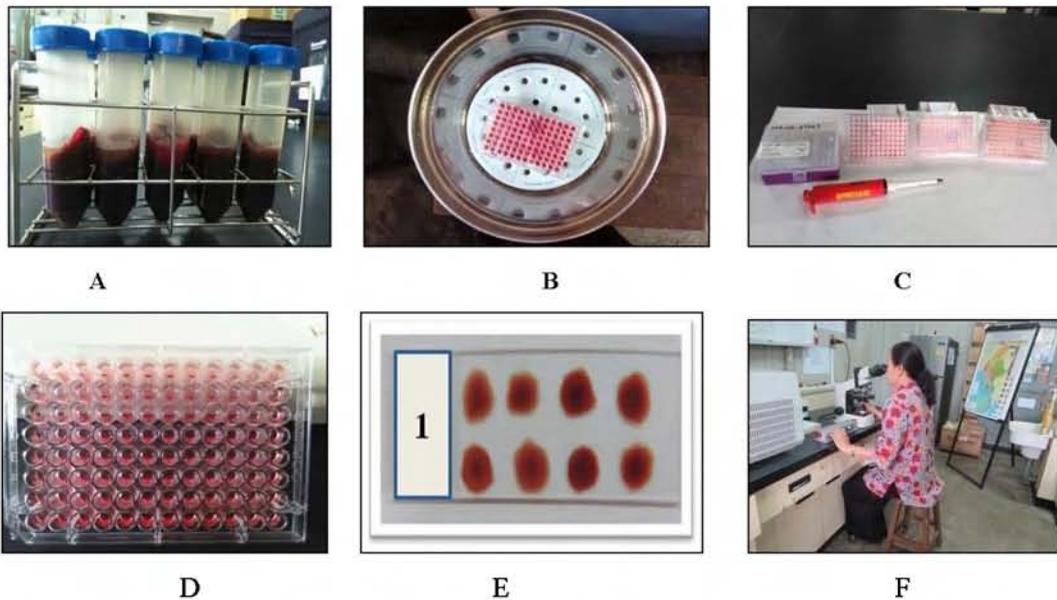


Figure 2. Photographs for screening of Antimalarial activity *Plasmodium falciparum*

- A. Blood Samples were placed in tube
- B. Incubation at 37°C for 30 hours
- C-D. Removed the blood supernatant
- E. After incubation for 30 hours, cultured blood were made thick blood films for parasite counting
- F. Counting Schizont and Trophozoite of *Plasmodium falciparum* by using Microscope

III. Results

Morphological characters of five medicinal plants

- Scientific name : *Samanea saman* (Jacq.) Merr.
- Myanmar name : Kala-kokko or Thin-baw kokko
- Family : Mimosaceae
- Part Used : Leaves

Outstanding characters of *Samanea saman* (Jacq.) Merr.

A large tree expended crown 30-80 feet high. Leaves alternate bipinnately compound, paripinnate, stipules linear lanceolate, pubescent, pulvinii very distinct; petiole cylindrical, canaliculate on upper surface; primary rachis canaliculated on upper surface, tomentose; secondary rachis long, pubescent; pulvinii very distinct, tomentose; leaflets oblique, ovate-oblong, accrescent toward the top of the pinna. Inflorescences terminal and axillary, pinkish, grouped in heads, the peduncles pubescent. Flowers dimorphic, with larger single central and many smaller laterals bisporangiate. Central flower solitary, larger, complete, embroctate, bracteates, the bracts ovate, pubescent, sessile; calyx of 5 united

sepals, tubular, pubescent outside, green, acute; corolla of 5 united petals, tubular, pink, acute, pubescent outside; androecium of 55 united stamens, monadelphous, glabrous, the filaments long, the anthers oblongoid, ditheous, dorsifixed, longitudinal dehiscence, extrorse; gynaecium of one carpel, the gynophore present, glabrous, with two glands at the base, the ovary superior, oblongoid, unilocular, marginal placentation, the style long, the stigma simple. Fruits legume straight or slightly curved, brownish-black, indehiscent, thick, fleshy and compressed, glabrous, septate between the seeds, ovoids, brownish - black, compressed. Winged seeds, flowering and fruiting time June to November (Figures 3-9).



Figure 3. Habit
Inflorescences



Figure 4. Upper and lower surface of leaves

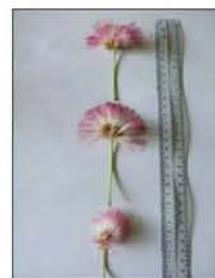


Figure 5.



Figure 6. Calyx
Ovary



Figure 7. Corolla



Figure 8. Androecium



Figure 9. T.S of

Scientific name : *Dactyloctenium aegyptium* (L.) Willd.

Myanmar name : Myet-thone-kha, Didok-chi

Family : Poaceae

Part Used : The Whole Plant

Outstanding characters of *Dactyloctenium aegyptium* (L.) Willd.

Moderately robust short-lived perennials; culms about 4.5 m tall, slender. Leaf sheaths ciliate at margin; ligule shortly fringed, ciliate membrane; leaf blades linear-lanceolate, flat, stiff, the base obtuse, the margin smooth, the tip acute to acuminate. Inflorescence digitately arranged, spikes 2-5 narrowly oblong, spreading. Spikelet broadly ovate; florets 4-5; the uppermost abortive. Glumes dissimilar, membranous, nerve of both glumes extended into stout scabrid awn; lower glumes broadly ovate, 1/2 as long as the lowest lemma, keel thick with hispidulous wing; upper glume obovate, keel smooth; lemmas and paleas membranous to hyaline, lemmas broadly ovate, keel gibbous, acuminate to short awns; paleas ovate, 2-nerved, keel winged, ciliolate. Stamens 2-3, anthers creamish. Stigmas white. Grain broadly obovate, coarsely transversely rugose. Flowering time from June to September (Figures 10-14).



Figure 10. Habit Figure 11. Inflorescence Figure 12. Spikelet Figure 13. Ligume Figure 14. Opened floret

Scientific name : *Brucea javanica* (L.) Merr.

Myanmar name : Yadan-zi

Family : Simaroubaceae

Part Used : Seeds

Outstanding characters of *Brucea javanica* (L.) Merr.

A shrub or small tree is up to 10m tall with soft-haired twigs and leaves. Leaves alternate, compound-imparipinnate; leaflets opposite, penni-veined, hair below, margin toothed. Inflorescence axillary, pubescent, composed of small cymes, united into bracteate, raceme-like thyrses. Flower minute, greenish-white to greenish-red or purple. Sepals is 4, connate at the base; petals 4, villous, glandular at the tip. Male flowers, stamens 4, pistil reduced to a stigma. Female flowers, stamens 4, much reduced. Ovary is with 4 free carples. Fruits drupe ovoid, black when ripe. Seeds compressed, rugose, blackish brown (Figures 15-18).



Figure 15. Habit Figure 16. Upper and lower surface of leaves Figure 17. Fruits Figure 18. Seeds

Scientific name : *Plumeria alba* Linn.

Myanmar name : Tayok-saga-aphyu

English name : Pagoda Tree or Temple Tree

Family : Apocynaceae

Part Used : Roots

Outstanding characters of *Plumeria alba* Linn.

Small trees, 10-30 feet high, lacticiferous; stems terete, Leaves alternate, spiralled at the end of the branches, elliptical, unicostate, coriaceous, both the surfaces glabrous; petioles cylindrical, longitudinal grooves in the ventral sides, glabrous; exstipulate. Inflorescences terminal cincinni, the peduncles glabrous, red; bracts broadly ovate, glabrous, deciduous.

Flowers white with yellow centre within, pink tinge without, actinomorphic, fragrant, the pedicels long, glabrous, ebracteolate; calyx 5, fused, campanulate, the lobes ovate, red, glabrous, persistent; corolla 5, salverform, hairy within, the lobes broadly elliptical, twisted, glabrous; stamens 5, epipetalous, inserted, the filaments long, the anthers ditheous, dorsifixed, longitudinal dehiscence, introrse; ovary half inferior, obovoid, free, 2-celled with numerous ovules in each cell, axile placentation, the style long, the stigma calyptriform. Fruits are cylindrical pods that are rarely formed in cultivation. Flowering from March to July (Figures 19-25).



Figure 19. Habit
Inflorescence

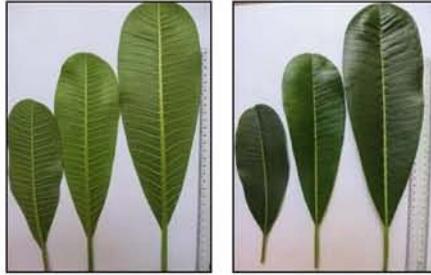


Figure 20. Upper and lower surface of Leaves



Figure 22. Calyx



Figure 23. Corolla

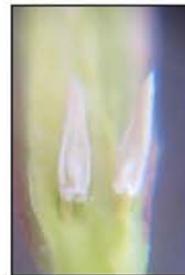


Figure 24. Androecium



Figure 25. Gynaecium

Scientific name : *Ferula foetida* Regal.

Myanmar name : Shein-go

English name : Asafoetida

Family : Apiaceae

Part Used : Gum-resin

Outstanding characters of *Ferula foetida* Regal.

Perennial herbs, 6-10 feet high, large fleshy root covered with bristly fibres, numerous stem, leaves with wide sheathing petioles, upper leaves divided into segments and young leaves are densely hairy. Flowers are hermaphrodite; appear in large terminal clusters, small and pale greenish yellow in colour. Fruit oval, flat thin, foliaceous, reddish brown with pronounced vittae, it has a milky juice and strong foetid odour, 1cm long, roots are robust carrot-shaped. Flowering time is from March to April (Figures 26-28).

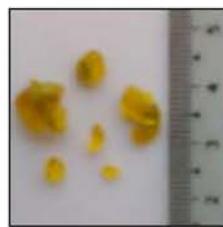
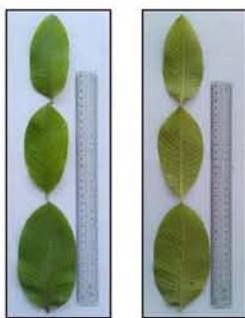


Figure 26. Habit Figure 27. Upper and lower surface of leaves Figure 28. Gum-resin

Table.1 Sensory characters of the five medicinal plants

Medicinal Plants	Parts Used	Sensory Characters			
		Colour	Taste	Odour	Texture
<i>Samanea saman</i> (Jacq.) Merr.	Leaves	Greenish	Bitter	Aromatic	Granular
<i>Dactyloctenium aegyptium</i> (L.) Willd.	The whole plant	Pale green	Bitter	Unpleasant	Granular and Fibres
<i>Brucea javanica</i> (L.) Merr.	Seeds	Dark brown	Bitter	Aromatic	Granular
<i>Plumeria alba</i> Linn.	Roots	Browish	Bitter	Fragrant	Granular
<i>Ferula foetida</i> Regal.	Gum-resin	Pale yellow	Bitter and Acrid or Nauseating	Fetid or Peculiar	Granular

Macroscopical characters of the five medicinal plants

Samanea saman (Jacq.) Merr. (Leaves) - Alternate, bipinnately compound, paripinnate, pulvinii very distinct; primary rachis canaliculated on upper surface, tomentose; secondary rachis long, pubescent.

Dactyloctenium aegyptium (L.) Willd. (The Whole Plant) - Moderately robust short-lived perennials; culms about 4.5 m tall, slender. Leaf sheaths ciliate at margin.

Brucea javanica (L.) Merr. (Seeds) - Seeds compressed, rugose, blackish brown.

Plumeria alba Linn. (Roots) – Large fleshy root, brownish colour

Ferula foetida Regal. (Gum-resin) - Obtained from incisions in the stem, rhizome and root of plant, milky liquid oozes out, the liquid then dries to form a resin, pale yellow colour.

Diagnostic characters of the combination of powdered of five medicinal plants

Diagnostic characters study of the combination of five medicinal plants of powdered pitted and simple vessels, fibres, fibre-tracheids, tracheids, laticiferous cells, oil cells and the fragments of epidermal cells. The results were shown in Fig 29.

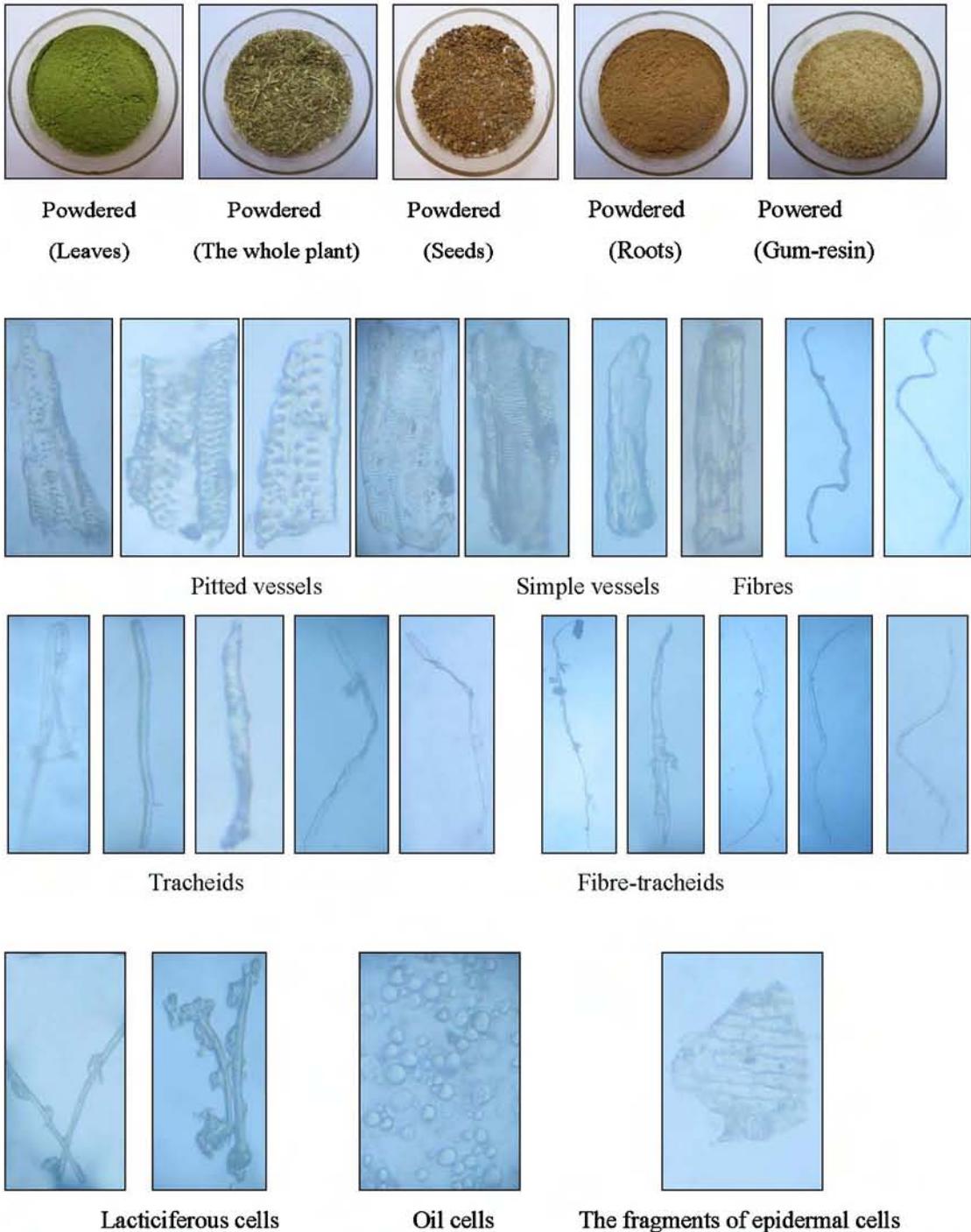


Figure 29. Diagnostic characters of the combination of powdered of five medicinal plants

Physicochemical properties of the combination of powdered of five medicinal plants

In physicochemical properties, the moisture content was usually determined by drying to constant weight and taking the loss in weight as moisture, total ash, acid insoluble ash, and

water soluble ash content were also recorded. All these values were useful for the quality control system regarding ash and it was used for medicinal purposes. The solubilities of combination of five medicinal plants powdered in petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol and distilled water were carried out to determine the amount of total solids soluble in an individual. The combination of five medicinal plants powdered was found to be significantly soluble in methanol, distilled water and ethanol than in other solvents. The results were shown in Table 2.

Table 2. Physicochemical properties of the combination of five medicinal plants powdered

No.	Physicochemical Characters	Combination of five medicinal plants powdered (Average %)
1	Moisture content	8.28
2	Total Ash	10.25
3	Acid-insoluble ash	25.43
4	Water-soluble ash	29.27
5	Petroleum ether soluble	6.35
6	Chloroform soluble	9.23
7	Ethyl acetate soluble	10.8
8	Acetone soluble	7.65
9	Methanol soluble	26.9
10	Ethanol soluble	18.66
11	D/W soluble	20.33

Table 3-4. *In Vitro* antimalarial effect of methanolic extracts of combination of five medicinal plants on *Plasmodium falciparum*

Study Area	Year	Drug
Shwe-kyin Township	2015	Combination of five medicinal plants extract

Microlitre Plate	A	B	C	D	E	F	G	H
Conc: Schizonts	Control Schiz	Conc: Schiz						
Drug conc: ($\mu\text{g/ml}$)	0	6.25	12.5	25	50	100	200	400

Table 5. Twenty person of criteria matched blood sample were tested *in vitro* assay

Sr. no.	A	B	C	D	E	F	G	H
	Control	6.25	12.5	25	50	100	200	400
1	38	29	21	17	10	0	0	0
2	29	20	17	13	7	0	0	0
3	35	29	20	14	6	0	0	0
4	58	47	32	21	13	7	0	0
5	41	36	27	19	11	4	0	0
6	37	31	25	19	10	4	0	0
7	63	57	41	27	13	5	0	0
8	40	31	25	19	10	4	0	0
9	31	27	20	16	9	3	0	0
10	53	46	35	19	7	2	0	0
11	50	42	30	26	12	4	0	0
12	34	29	20	13	4	0	0	0
13	37	33	26	17	11	4	0	0
14	51	46	32	21	15	9	0	0
15	31	27	20	14	8	2	0	0
16	34	29	15	10	3	0	0	0
17	51	42	32	20	11	4	0	0
18	27	20	13	9	4	0	0	0
19	41	36	30	23	17	11	5	0
20	63	51	42	29	11	0	0	0

Table 6. Schizont Maturation Inhibitory (%)

Drug concentration	SMI %
6.25	16.69
12.5	38.63
25	57.12
50	76.01
100	90.73
200	99.39
400	100.00

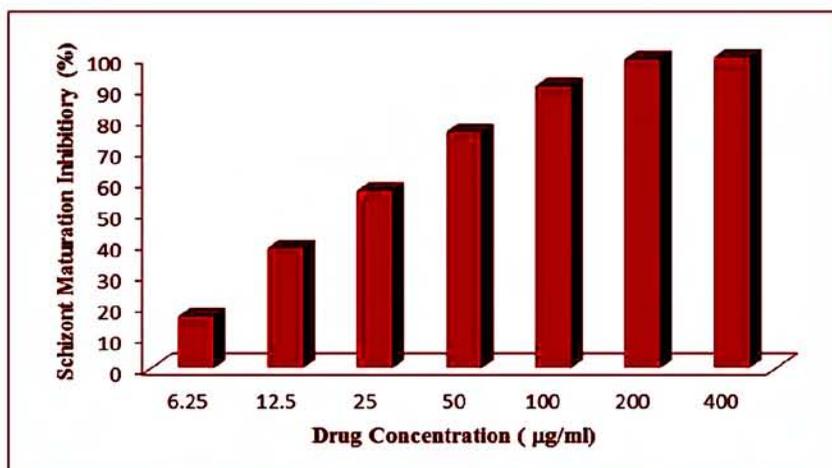


Figure 30. Schizont Maturation Inhibitory (%) of 20 no. Patients blood sample treated with control groups against *Plasmodium falciparum* in vitro test

The concentration of drugs that produce a 50% decrease parasitaemia compared to the negative control culture referred as 100% parasitaemia. Methanolic extracts of combination of five medicinal plants showed EC_{50} value 19.44µg/ml. Therefore this methanolic extracts showed highly active antimalarial effect on *Plasmodium falciparum*. The results were showed in Table.7 and Fig.31.

Table 7. Antimalaria activity of combination of five medicinal plants against *Plasmodium falciparum in vitro* tes

EC	Mean	95% Confidense Intervals	
		Lower	Higher
EC ₁	1.2566	0.5141	3.0713
EC ₁₆	6.0291	4.1952	8.6648
EC₅₀	19.4459	13.5309	27.9468
EC ₈₄	63.7194	43.6414	90.1373
EC ₉₀	87.9469	49.2709	156.9822
EC ₉₅	134.8984	68.4402	265.8903
EC ₉₉	300.9296	123.1200	735.5316

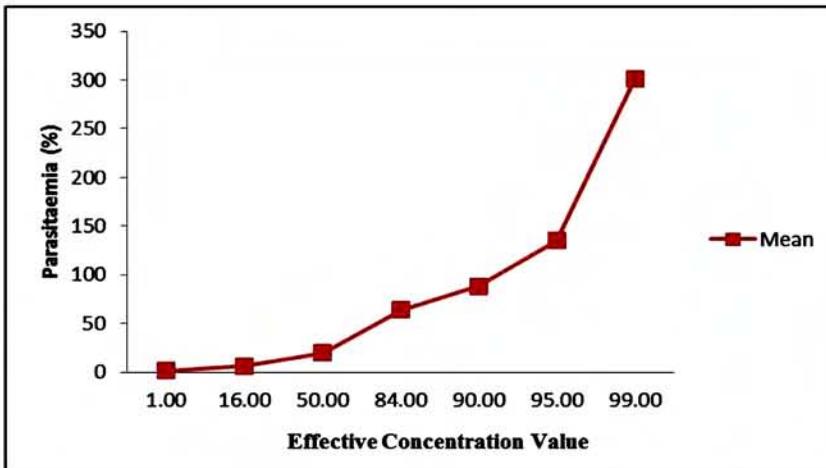


Figure 31. Mean Parasitaemia (%) of 20 no. of Patients blood sample treated with control groups against *Plasmodium falciparum in vitro* test

IV. Discussion and Conclusion

In this paper, morphological characters, the sensory characters, macroscopical and diagnostic characters of the combination of powdered of five medicinal plants; the physicochemical properties of the combination of powdered of five medicinal plants and antimalarial effect from combination of five medicinal plants extract were carried out.

According to Bailey, 1961; Backer, 1963, Hutchison, 1964 and Thet Thet May, 1986 the *Samanea saman* (Jacq.) Merr. plant is 60-80 feet, with expanded crown, unarmed. The leaves are bipinnately compound. The inflorescences were globose or sub-globose heads having 20-30 flowers, the peduncle long. Flowers bracteates, complete, bisexual, hypogynous and pink in colour.

Khare, 2007 showed that *Dactyloctenium aegyptium* (L.) Willd. plant is culms moderately robust, leaf sheath ciliate at margins, 2-5 digitately spreading narrowly oblong spikes; glumes dissimilar, membranous, nerves of both glumes extended into stout scabrid; lower glume keel thick with hispidulous wing; stamens 2-3 and coarsely transversely rugose grain are characters of the species.

Padua, 1999 and Prajapati, 2003 reported that *Brucea javanica* (L.) Merr. plant is a monoecious or dioecious shrub or small tree up to 10 m tall with soft-haired twigs and dioecious. Drupe ovoid black when eaves. Leaves arranged spirally, imparipinnate, 20-50 cm long, exstipulate, leaflets 8-15, opposite, short petiolate, ovate-oblong to ovate-lanceolate, margin bluntly serrate or crenate, secondary veins unbranched and terminating in a marginal gland. Inflorescence axillary, pubescent, composed of small cymes united into bracteate, raceme-like thyrses. Flowers unisexual, 4-merous, small, greenish-white to greenish-red or purple; sepals connate at base; petals free; disk intrastaminal, thick, 4-lobed; stamens short, vestigial or absent in female flowers; ovaries superior, free, each with a single, pendent ovule, styles free or coherent at base, subulate, bent outwards over the top of the ovary. Fruit consist of 1-4 hardly, fleshy drupelets; 2-ribbed; dry, purplish black and 4-5 (-7) mm long when mature, pyrene with wrinkled endocarp. Seed compressed rugose, blackish-brown, ovoids, with thin testa and very thin endosperm.

The Wealth of India, 1969 stated that *Plumeria alba* L. plant is the distinguishing features of this species are the elliptical leaves with acuminate tips and the flowers with broadly elliptical lobes tinged with pink colour externally. The trees are with stout but weak and fleshy branches and milky juice. The leaves are large, lanceolate, narrow and spirally arranged near the ends of branches, 20-40 cm long. They have waxy funnel shaped highly fragrant flowers; grow in clusters at the tips of the branches. It is native to warm America.

According to the Wealth of India, 1956 *Ferula foetida* Regal, a genus of perennial herbs, 6-10 feet high, large fleshy root covered with bristly fibres, numerous stem leaves with wide sheathing petioles, upper leaves divided into segments and young leaves are densely hairy, flowers pale greeny yellow. There are a number of schizogenous ducts in the cortex of the stem, full of the regious gum, on incision, the resin flows out. Compound large umbels arise from large sheaths. Some of the species are important as sources of oleo gum-resins used in medicine and as condiment.

The combination of powdered of five medicinal plants are simple and pitted vessels, fibres, tracheids, fibre-tracheids, lacticiferous cells, oil cells and the fragments of epidermal cells are also observed. These characters are in accordance with (Metcalf and Chalk, 1950 and 1960).

In the study of physicochemical properties, moisture contents, ash content and different solubility of organic constituents in organic solvents were determined. The solubility of combination of powdered of five medicinal plants was found to be significantly soluble in methanol, distilled water were found to be the greatest than in other solvents. These characters are in agreement with (The British Pharmacopoeia, 1968).

In Myanmar, Traditional practitioners used combination of five medicinal plants (TMF-58) as antimalarial herbs traditionally in many regions but there were no scientific exploration concerned with their effectiveness.

So, in this research ant malarial effect of *Samanea saman* (Jacq.) Merr. (Kalar-kokko); *Dactyloctenium aegyptium* (L.) Willd. (Myet-thone-kha); *Brucea javanica* (L.) Merr.

(Yadan-zi); *Plumeria alba* L. (Tayok-saga-aphyu) and *Ferula foetida* Regal. (Shein-go) were scientifically investigated for the first time in Myanmar.

According to Thet Thet May, 1986 *Samanea saman* (Jacq.) Merr. were proved antimalaria activity against *Plasmodium falciparum* from 5 human patients could be detected using the *in vitro* microtest method for sensitivity of *Plasmodium falciparum* to antimalarial drugs.

Brucea javanica (L.) Merr. herb can produce better effects with tertian malaria and quartan malaria and it is also effective against pernicious malaria (<http://www.e2121.com/herb-db/viewherb.php.3?Viewed=179>). Padua, 1999; Khare, 2007 showed that Quassinoids (eg. Brusatol) from the fruits, as well as the triterpenoids bruceajavanin A, dihydrobruceajavanin A and bruceajavanin B (from the stems of *B. javanica*) have been shown to inhibit the growth of the chloroquine-resistant strain *Plasmodium falciparum* KI *in vitro*. Some of the quassinoids (bruceine A, B and D, brusatol) also showed *in vivo* activity against *Plasmodium berghei* infections in mice after oral dosing. Finally, the quassinoids bruceine A, B and C, present in a chloroform extract of *Brucea javanica* fruits had a very potent activity *in vitro* against a multi-drug resistant *Plasmodium falciparum* strain with an ID₅₀ of 8.66, 6.15 and 1.95 ng/ml, and the mefloquine reference.

Ferula foetida Regal. traditionally, a daily dosage of asafetida resin 200-500 mg is used for medicinal purpose (<http://www.drugs.com/npp/asafetida.html>).

The antimalarial properties of *Plumeria alba* L. were probed using aqueous (30-300 mg/kg) and dichloromethane/methanol (30-300 mg/kg) extracts of the plant in imprinting control region mice infected with *Plasmodium berghei*. For the curative test, the extracts were administered to the infected mice 4 days post-infection. In the prophylactic test, the animals were pre-treated with the extracts for 3 days before challenging them with *Plasmodium berghei* infected erythrocytes. The aqueous extract significant (P<0.05-0.001) effects on *Plasmodium berghei* infection is similar to artemether and lumefantrine curatively and sulfadoxine/pyrimethamine prophylactically. However, the dichloromethane/methanol extract reduced the parasitaemia curative (P<0.05-0.01) but not prophylactically. This study provides evidence to support the antimalarial properties of stem bark extract *Plumeria alba* L. in mice ([Http://www.doverpress.com/invivo-antimalarial-activity-of-stem-bark-extracts-of-plumeria-alba-a-peer-reviewed-artice-RIP](http://www.doverpress.com/invivo-antimalarial-activity-of-stem-bark-extracts-of-plumeria-alba-a-peer-reviewed-artice-RIP)).

Desjardins, 1979 described that measured titrated hypoxanthine incorporation and the emphasis of final estimate of susceptibility shifted to the EC₅₀ which was defined as the concentration of a drug required to inhibit parasite growth by 50% comparing with the same sample grown without drug, is now the standard measure of antimalarial *in vitro* sensitivity.

To find out the effect of combination of five medicinal plants extract on human malaria parasite, *Plasmodium falciparum*, *in vitro* sensitivity assay was carried out. Regarding with the data from *in vitro* assay, EC₅₀ representing the concentration of culture which was referred as 100% parasitaemia. EC₅₀ value of methanolic extracts of combination of five medicinal plants is 19.44µg/ml. Therefore this methanolic extracts showed highly active antimalarial activity on *Plasmodium falciparum*. Our finding confirms the importance of investigating the antimalarial effect of plant species used in traditional medicine. So, further study on *in vivo* antimalarial activity on *Plasmodium berghei* and toxicity test should be performed.

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