Understanding of Malaria and Its Therapeutic Regimens–The Way Forward

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Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

ABSTRACT

Aim: Malaria is a very serious deadly disease that has attracted the attention of many researchers all over the world. Because a lot of work has been done in the area of malariology, there is need to understand its advance pattern and therapeutic regimens.

Methods: Past and recent literatures on malaria were searched for information on history, global situation, classification, biology, pathology, pathogenesis, diagnosis, treatment and control of malaria to assess the progress made so far in the area of malariology.

Results: Malaria is an ancient disease recognized by Hippocrates over 2413 years ago, caused by Plasmodium species, first identified by Charles-laveran 123 years ago affect 300–500 millions human worldwide, responsible for 3 deaths in every 30 seconds. The knowledge of classification, biology, pathology, pathogenesis, diagnosis and treatment of malaria is a tremendous achievement towards the control of the disease.

Conclusion: But complete elimination of malaria perhaps will still take another time, since lots need to be known about the molecular biology of antigenic shift and drift, nature and mechanisms of action of the parasite toxin, in order to have basis for definite vaccine development. By so doing, radical cure and total eradication of malaria can be achieved.

Keywords: Malaria; hippocrates; laveran; antigenic shift; radical cure; eradication.
1. INTRODUCTION

Malaria is an infectious disease caused by a protozoan of the phylum: Apicomplexa, class: Sporozoea, subclass: Coccidia, suborder: Haemosporina, family: Plasmodidae, genus Plasmodium and transmitted by the female mosquito of the genus Anopheles when it feeds by sucking human blood and whose life cycle alternates between man and mosquito.[1] An anopheline mosquito inoculates plasmodium sporozoites to initiate human infection. Circulating sporozoites rapidly invade liver cells. Exoerythrocytic stage tissue schizonts mature in the liver and invade erythrocytes. Only erythrocytic parasites cause clinical illness. Repeated cycles of infection can lead to the infection of many erythrocytes and serious disease. Sexual stage gametocytes also develop in erythrocytes before taken by mosquitoes where they develop into infective sporozoites [2,3]. The means of infection are through infective mosquito bites, contaminated syringes and vertical transmission from mother to foetus through placenta [4,5]. Malnutrition, splenomegaly, and anaemia are the expected complications of repeated attacks of malaria [1,2].

The emergence of resistant malaria in addition to the toxicity of drugs currently used as anti-malarials has created a major concern and an urgent need for development of new anti-malarial agents. Throughout the world, herbs have sustained man not only as source of food but also as medicines utilized in various ways for varied purposes. In the last decade, people have become increasingly aware of the use of medicinal plants for the treatment of diseases [6]. In developing countries, it is important to know the therapeutic potentials of substances from natural sources, since local drugs could be of great value and substitute for the more sophisticated and expensive drugs. Therefore, there is need to find cheaper and pharmacologically active substances from natural products and this has defined the research goals of developing nations as encouraged and recommended by the World Health Organization [7].

Traditional healers have made various efforts to control diseases including malaria-using herbs. Few of these plants have been properly identified and documented. Only a very small percentage of the plants with ethnopharmaceutical potentials have been subjected to scientific analysis hence their safety and efficacy are questionable [8]. World Health Organization (WHO) in recognition of the increased value of herbal medicine in primary health care, especially in developing countries has advocated for the proper identification, sustainable exploitation, scientific development and appropriate utilization of herbal medicines, which provide safe and effective remedies in medicine [9]. Investigation on the risks associated with prolonged and improper use of some herbal drugs revealed a potential for toxic and teratogenic effects in biosystem [10], yet scientific information and the results of comparative studies are scarce on the effectiveness and safety of most traditional remedies and techniques [11]. Of the estimated 300,000 plants species acclaimed worldwide as possible pharmaceutical candidates, only about 5% have been investigated scientifically for medicinal properties [8].

2. JUSTIFICATION

Malaria is the worst human problem at the moment. There were an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly children under 5 years and 109 countries were endemic for malaria in 2008, but 45 countries are within African region. More than 1 reported cases per 1000 population per year was reported in 2006. Nigeria accounts for a quarter of all malaria cases in the African region and almost all cases are caused by P. falciparum with recorded 57,506,430 malaria
cases and 225,424 deaths in 2006. In 2007, Nigeria was the 7th malaria country. Treatment is recommended for malaria control [12]. The disease causes death and suffering, financial hardship and retards economic growth and improvement in living standard [13]. Between 10 and 13% of maternal deaths are caused by malaria in endemic countries and spontaneous abortions are reported in up to 60% of maternal malaria cases [14]. The rapid spread of resistance to antimalarial drugs present a potentially devastating threat to effective, safe and affordable treatment. Chloroquine was the main drug used for decades, but increasing resistance forced its replacement with other antimalarial drugs [13], 40–60% of malaria cases in Nigeria have been reported not to respond to treatment with the drug [15]. Toxicity of antimalarial drugs and lack of vaccine against malaria have compounded problems in malaria chemotherapy [15]. While large number of people in the rural areas of developing countries still consume crude decoctions and concoctions of medicinal herbs [16]. Scientific and technological advances in the field of chemotherapy will boost malaria control [17].

Since prehistoric times, plants based medicines have been in use for treatment of diseases until synthetic drugs were developed in the 19th century. Today the use of herbs continue to exist throughout the developing world, while 40% of prescription drugs are still herb based remedies for treatment of illnesses [18,19]. Phytochemicals are protective, disease preventing plant substances [20]. Natural products in several developing countries are still the mainstay of all medicines [21]. The most commonly used sources of drugs are herbs, plant extracts, seeds, leaves, barks of certain plants, tubers and roots. Noteworthy in this regard is the use of single remedy or plant extract to treat more than one disease and combination of various plants extracts for broad-spectrum therapy. Within the context of traditional practice, decoctions or infusions of plants have been used commonly to treat malaria and malaria symptoms. There are numerous illustrations of plant based antimalaria drugs. Quinine is an important drug of plant origin with history of long use. This alkaloid occurs naturally in the bark of Cinchona tree, and is used in the treatment of malaria apart from its use to relieve nocturnal leg cramps [22]. Artemisia annua has been in use for more than 2000 years and is found effective in the treatment of malaria [23]. The plant, Azadirachta indica has been used extensively for malaria treatment in Nigeria and was proven to have antimalarial activity [24].

Other plants that have been used include; Sida acuta, Carica papaya, Vernonia amygdalina, Khaya senegalensis, Nuclea latifolia, Anacardium occidentale, Mangifera indica, Sclerocarya birrea, Spondia mombin, Balanites aegyptica, Newboldia laevis, Commiphora kerstingii, Allium sativum, Tridilia emetica, Phoenix dactylifera, Piper guinense, Neocarya macrophylla [25], Achillea millefolium, Baccariss trimeria, Lippia alba, Matricaria chamomilla, Mikania glomerata, Tanacetum pathenium [26], Duguetia furfuracea, Xylopia emarginata [27], Centella asiatica [28], Bupleurum montanum, Bupleurum plantagineum [29], Ferula opoda [30], Cynodon dactylon [31], Setaria magaphylla [32], Momordica foetida [33], Momordica charantia, Curcubita maxima [34], Phyllanthus amarus and Phyllanthus niruri [35]. The alkaloids lysicamine, trivalvone, palmatine, jatrorrhizine and columbamine from Annickia kummeriae showed strong to moderate antiplasmodial activities with IC_{50} value of 2.8–14.3µg/ml) [36]. Two stilbenes, longistylin A and C and betulinic acid from Cajanus cajan showed a moderately high in vitro activity against P. falciparum strain 3D7 [37]. Tropical plants that are used for the treatment of malaria in over 100 underdeveloped and developed countries of the world are provided in Table 1.
Table 1. Plants used for Anti-malaria purpose in Central Tropical Africa

| Plant material, family, genus, species & English names | Vernacular names | Plant part (s) used | Therapeutic regimen (s) | Pharmacologic/ toxic principle (s) |
|-------------------------------------------------------|----------------|-------------------|------------------------|-----------------------------------|
| **Amaranthaceae**<br*Achyranthes aspera* (Devils horsewhip) | Hakorinmaciji (H) Aburo (Y) Egyagi (N) | Whole plant | Decoction taken once: Steam relief headache | Alkaloid, potassium salt |
| **Amaranthaceae**<br*Alternanthera sessilis* (Sessile joyweed) | Maikaidubu (H) Masantogi (N) Dagunro (Y) | Whole plant | Decoction taken orally | Alkaloids |
| **Amaranthaceae**<br*Pupalia lappacea* | Marin kusu (H) Emaagbo (I) Mamarigbo (N) Ose (Y) | Whole plant | Leaf or root decoction taken orally | - |
| **Poaceae**<br*Elevisine indica* (Goose grass) | Ciyawaruji (H) Chinchere (N) Ese-Kannakanna (Y) | Whole plant | Make decoction and take once daily | Elevisine |
| **Guttiferae**<br*Garinia kola* (Bitter kola) | Namijin goro (H) Akilu (I) Ewogichi (N) Orogbo (Y) | Seed, stem root | The powder is taken: It can be chewed also | Kolavirone, ametoflavine, tannins |
| **Irvingiaceae**<br*Irvingia gabonensis* (wild mango) | Goron biri (H) Ogbono (I) Kpeakpea (N) Eso oro (Y) | Leaves | Leaf decoction is made and taken | Protein, fat |
| **Labiatae**<br*Ocimum basilicum* | - | Leaves | Cold water maceration is taken | - |
| **Anacardaceae**<br*Anacardium occidentalis* (Cashew) | Kashew (H) Okpokopo (I) Kashiwu (N) Kaju (Y) | Stem bark | Decoction taken once daily | Cardol, phenol, gallic acids, resorcinol, anacardic acid |
| **Anacardaceae**<br*Mangifera indica* | Mangwaro (H) Mangolo (I) | Leave, stem bark | Decoction is made with other additives and taken | Resins, glycoside, quercetin, flavonoids |
| Family            | Common Name                          | Local Names                | Preparation                                                                 | Remarks                                                                                   |
|-------------------|--------------------------------------|---------------------------|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Anacardaceae      | Mangoro (N)                           | Dinyà (H)                 | Dried stem bark decoction is taken twice daily                            | Tannins                                                                                 |
| Anacardaceae      | Spondias mombin (Hog plum)           | Tsadar masar (H)          | Decoction or infusion is taken daily for 1-7 days                         | Geraniin, galloylgeraniin, geraniin, galloylgeraniin                                       |
| Annonaceae        | Carissa edulis                        | Uwar banza (H)            | Root decoction is taken once daily                                        | -                                                                                        |
| Annonaceae        | Crossopteyx febrifuga                 | Kasiya (H)                | Decoction from twigs leaf is taken orally                                 | Crossptine, phytosterol, pholobaphene β-quinoine                                           |
| Apocynaceae       | Rauwolfia vomitoria                   | Wadda (H)                 | Infusion is taken daily; leaf powder is taken with porridge               | Rauwolfine, reserpine, rescinnamine, aijimaline, alstonine, yohimbine, saposterol, serpentine |
| Apocynaceae       | Alstonia boonei                       | Ahun (Y)                  | Make decoction and take twice daily                                       | -                                                                                        |
| Asteraceae/Compositae | Chromolaena odorata (Siam weed)       | Ekpe gbakun (N)           | Make decoction with potash and take once daily                             | Pinene, limonene, candinene, flavonoids                                                  |
| Asteraceae        | Acanthospermum hispidus (Star bur)    | -                         | Juice from the leaves with potash once daily                              | Acanthospermolides, inulin                                                               |
| Asteraceae        | Chrysanthemum indicum                 | Rariya kasa (H)           | Decoction is taken once daily                                             | -                                                                                        |
| Combretaceae      | Tridax procumbens (Tridax)            | -                         | Cold water infusion with potash is taken                                  | -                                                                                        |
| Compositae/Asteraceae | Vernonia amygdalina (Bitter leaf)    | Tsula (N)                 | Leaf infusion or stem bark decoction is taken daily                       | Vernodalin, vernomygdin, vernolepin, vernolide, flavonoid                                 |
| Compositae/Asteraceae | Vernonia amygdalina (Bitter leaf)    | Shiwaka (H)               | Leaf infusion or stem bark decoction is taken daily                       | Vernodalin, vernomygdin, vernolepin, vernolide, flavonoid                                 |
| Compositae/Asteraceae | Vernonia amygdalina (Bitter leaf)    | Ewuro (Y)                 | Leaf infusion or stem bark decoction is taken daily                       | Vernodalin, vernomygdin, vernolepin, vernolide, flavonoid                                 |
| Compositae/Asteraceae | Vernonia amygdalina (Bitter leaf)    | Olubo (I)                 | Leaf infusion or stem bark decoction is taken daily                       | Vernodalin, vernomygdin, vernolepin, vernolide, flavonoid                                 |
| Plant Class          | Species                                      | Common Names                                      | Parts Used                  | Preparation            | Active Constituents                                                                 |
|----------------------|----------------------------------------------|---------------------------------------------------|-----------------------------|------------------------|------------------------------------------------------------------------------------|
| **Asteraceae**       | *Vernonia cinerea* (little iron weed)        | Lupeol acetate, vernomoside, β-amyrin, sesquiterpine, β-sitosterol, pene lactine, stigmasterol, luperol, α-spinasterol |
| **Asteraceae**       | *Compositae/Asteraceae Vernonia perrottetii* | Whole plant, Decoction is taken orally             | Vernoniin                   |                        |                                                                                   |
| **Balanitaceae**     | *Balanites aegyptiaca* (Desert date)         | Aduwaa (H), Adua (N)                               | Bark, seed (it causes nephrosis and hepatitis) | Decoction of either bark or seed is taken 1-3 days | Disogenin, sapogenin, yamogenin, flavonoids, triterpenoid, sterols, carotenoids    |
| **Bignoniaceae**     | *Newboldia laevis* (Tree of life)            | Aduruku (H), Dinberechymile (N), Ogirisi (I), Akoro (Y) | Roots, leaves               | Infusion is taken daily | Alkaloids, tannins, flavonoids                                                    |
| **Bombacaceae**      | *Ceiba pentandra*                            | Rimin (H), Kada (H), Akpu (I), Kuchi (N), Ogungun (Y) | Leaves, barks, roots        | Decoction is taken oftenly         | -                                                                                  |
| **Boraginaceae**     | *Heliotropium indicum* (Wild clary)          | Karkashin korama (H), Etigulu (N), Ogbe-akuko (Y)  | Whole plant                 | Infusion or decoction taken daily | Indicine-N-oxide, saponins, tannins                                              |
| **Caesalpiniaceae**  | *Cassia occidentalis* (Negro coffee)         | Tafasar masar (H), Rere (Y), Gaya (N), Akede ogbara (I) | Leaves                      | Infusion is taken once daily      | Toxalbumin, anthracene glycoside, sennoides, flavonoids                            |
| **Caesalpiniaceae**  | *Chamacrostachys rotundifolia*               | Ilegbere (Y), Damma (N)                            | Whole plant                 | Decoction taken daily          | -                                                                                  |
| **Caesalpiniaceae**  | *Daniellia oliveri* (Ilorin balsam)          | Maje (H), Ozabwa (I), Danoli (N)                   | Stem bark, leaves, bark, leaf buds | Decoction taken daily; powder can also be taken | Alkaloids, tannins, gum                                                            |
| Family                  | Species                                    | Common Names                  | Parts Used                  | Preparation                          | Ingredients                                      | Medicinal Uses                                                                 |
|------------------------|--------------------------------------------|-------------------------------|-----------------------------|---------------------------------------|--------------------------------------------------|--------------------------------------------------------------------------------|
| Caesalpiniaceae        | Piliostigma thonningii (Thoning’s piliostigma) | Iya (Y)                      | Fresh leaves, dried stem bark, root bark, fruits | The decoction of the first 3 is taken; fruit powder infusion is taken daily | Alkaloid, tannin                                  |                                                                                  |
| Caesalpiniaceae        | Tamarindus indica (Indian tamarind)         | Kalgo (H)                     | Leaves, fruits, fresh stem bark | Decoction made with potash or cold water infusion is taken daily | Tartaric acid, citric acid, malic acid, tannins   |                                                                                  |
| Capparidaceae          | Cleome viscoe (Chicken weed)               | Tsamiya (H)                   | Whole plant                 | Decoction is taken orally             | -                                                |                                                                                  |
| Capparidaceae          | Cleome viscoe (Chicken weed)               | Egyagi (N)                    |                            |                                      |                                                  |                                                                                  |
| Caricaceae             | Carica papaya (Pawpaw)                     | Gwanda (H)                    | Seed, root                  | Cold water infusion or decoction is made. Half a cup is taken daily | Papain, cryptoxanthin, glycosides, polyphenols   |                                                                                  |
| Celastraceae           | Maytenus senegalensis                      | Namiji tsada (H)             | Roots, leaves               | Decoction or infusion is made and taken daily | Maystansine, flavonol                            |                                                                                  |
| Combretaceae           | Pteleopsis habeensis                       | Lallen giwa (H)              | Leaves                      | Decoction with Xylopia aethiopica and Capsicum frutescens taken orally | -                                                |                                                                                  |
| Canelliaceae           | Warburgia ugandensis (Fever tree)          | -                             | Dried stem bark, roots, leaves | Decoction or infusion is taken daily | Drimenol, warbugin, warburgiadiene, ugandesoline, muzigachial |                                                                                  |
| Solanaceae             | Withania somnifera                         | Karama anta (H)              | Leaves                      | Powdered leaf is taken; enema of decorticated root is used on infants | Withanine, withernolide, witherferin              |                                                                                  |
| Combretaceae           | Brysorcapus coccineus                      | Kimbar maharba (H)           | Roots, stem, leaves         | Cold water infusion of the root and that of lawsonia inermis; take a tumbler daily in the morning | Alkaloid                                         |                                                                                  |
| Ebanaceae              |                                          | Faru (H)                     | Root bark, stem             | Decoction is taken once               | Phembagin, scopolin,                              |                                                                                  |
| Family | Genus | Common Names | Parts Used | Preparation | Active Ingredients |
|--------|-------|--------------|------------|-------------|-------------------|
| **Euphorbiaceae** | *Euphorbiaceae* | *Alchornea cordifolia* | Leaves, stem bark, root | It is used to prepare malaria mixture with other leaves; taken once | Inulin, alchornin, tannin, alkaloid |
| **Euphorbiaceae** | *Euphorbiaceae* | *Jatropha curcas* | Fruits, leaves, root, seeds | Decoction is taken half tumbler daily | Curcin, inulin, tetramethyl-pyrazine, phobolesters |
| **Euphorbiaceae** | *Euphorbiaceae* | *Manihot esculentus* | Leaves | Decoction is taken once daily | Hydrocyanide, tannins, alkaloid, saponins |
| **Euphorbiaceae** | *Euphorbiaceae* | *Phyllanthus amarus* | Whole plant | Decoction is taken once daily | Tannins, saponins, inulin |
| **Fabaceae** | *Fabaceae* | *Xanthoxylum xanthoxyloides* | Roots, stem bark | Decoction is taken once daily | Alkaloids, vanillic acid, xanthoxyloid, 2-hydroxy benzoic acid |
| **Gramineae/Poaceae** | *Gramineae/Poaceae* | *Cympogon citratus* | Leaves | Tea is prepared and taken oftenly | Limonine, neryl, geraniol, citronellal, campene, triterpenes, flavonoids |
| **Lamiaceae** | *Lamiaceae* | *Ocimum gratissimum* | Leaves | Decoction taken thrice daily | Eugenol, thymol, camphor, carryophylline |
| **Liliaceae** | *Liliaceae* | *Allium cepa* | Bulb | The leaves are chewed for 10 days | Riboflavin, alliin, sulphur, allicin compounds |
| **Liliaceae** | *Liliaceae* | *Allium sativum* | Rhizomes | Decoction of fresh or dry leaves is taken for 10 days | Allicin, alliin, alliinase, flavonoides, cyanogenic glycoside |
| **Loganiaceae** | *Loganiaceae* | *Kookiyar* | Leaves, fruits | Infusion is taken once | Nigitanin, barterine, |
| Plant Family | Scientific Name | Common Name (N) / Local Name (H) | Part Used | Preparations | Active Constituents |
|-------------|-----------------|----------------------------------|----------|-------------|-------------------|
| **Leguminosae**<br>Entalda plaseoloides | - | Bark | Decoction is taken twice daily | - |  
| **Malvaceae**<br>Abelmonchus esculentus (Lady's finger) | Kpami (N) Kubewa (H) | Fresh fruits, leaves | - | Fernesol |  
| **Malvaceae**<br>Sida acuta | Sangii ekoti (N) | Whole herb | Decoction is taken orally | Alkaloid, flavonoid, prostaglandin, tannins |  
| **Ceratonia siliqua** (Carob bean) | - | Seeds | Molasses are extracted from seeds and taken accordingly | Molasses gum |  
| **Meliaceae**<br>Azadirachta indica (Neem tree) | Dogon yaro (H) | Stem bark, root | Decoction of stem bark or root is taken orally | Nimbin, nimbidion, nimbidin, salanin, meliacin |  
| **Meliaceae**<br>Khaya senegalensis (Mahogany) | Madaaci (H) | Stem bark, leaves | Decoction is taken twice daily | Scopoletin, sterol, limonoid gedunin |  
| **Meliaceae**<br>Trichila emetica | - | Stem bark, root, leaves | Decoction is taken once daily | - |  
| **Mimosasae**<br>Dichrostachys cinerea (Cow thorn) | - | Roots, fruits, leaves | A tumbler of hot decoction is taken daily | Tannins, alkaloids |  
| **Mimosasae**<br>Parkia biglobosa (Niffa) | Dorowa (H) | Leaves, stem bark, fruits | Make decoction and take once daily | Alkaloid, saponin, tannin |  
| **Moraceae**<br>Ficus thonnigii | - | Leaves, fruits | Decoction is taken once daily | - |  
| **Moraceae**<br>Musanga cercropioides (Umbrella tree) | - | Leaves, roots, stem | Decoction is taken orally | - |  
| **Myristicaceae**<br>Psidum guajava (Guava) | Gwaba (H) | Leaves | Decoction is taken daily | Quercetin, flavonoids, sapogenins, eugenol |  
| **Ochinaceae** | Namijin Kade (H), Stem, bark, root | The powder is taken with | - | Alkaloid, tannin, saponin, |  

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| Plant Family          | Species                                      | Common Names                                | Uses/Preparation                                                                 | Constituents                                      |
|----------------------|----------------------------------------------|---------------------------------------------|----------------------------------------------------------------------------------|--------------------------------------------------|
| **Lochira lanceolata** | (Iron wood)                                  | Okopia (L), Maganchi (N), Iponhon (T)       | meat when required                                                               | resin                                            |
| **Onagraceae**       | Ludwigia octovalris                          | Shashatau (H)                               | Whole plant                                                                      | Leaf decoction is taken once daily               |
| **Palmae**           | Cocos nucifera                               | Kwakwa (H)                                  | Bark, root, nut, leaves, fruit                                                  | Decoction or infusion of bark, rook, leaves is taken once daily |
| **Palmae**           | Phoenix dactylifera                          | Dabbino (H)                                 | Dried fruits                                                                     | The fruits are eaten                              |
| **Papilionaceae**    | Abrus precatorius                            | Idon zakara (H)                             | Leaves                                                                          | Cold water maceration or dried powder is taken for 3days |
| **Papilionaceae**    | Pterocarpus erinaceus                        | Madobiya (H)                                | Stem bark, leaves, fruits                                                       | Decoction or infusion taken 1-3 times daily      |
| **Papilionaceae**    | Tephrosia bracteolate                        | Samaci (H)                                  | Whole plant                                                                     | Decoction is taken once daily                    |
| **Piperaceae**       | Piper guinense                               | Masoro (H)                                  | Fruits, leaves                                                                  | They are used as adjuvants and taken per os       |
| **Rosaceae**         | Neocarys macrophylla                         | Gwaza (H)                                   | Fruit, kernel, twig                                                              | Decoction is taken orally                        |
| **Rubiaceae**        | Cryssopteryx tebrifuga                       | Kasiya (H)                                  | Root, twig, stem bark, leaf                                                      | Decoction is taken orally                        |
| **Rubiaceae**        | Nuclea latifolia                             | Tafashiya (H)                               | Root, fruit, stem bark                                                          | Decoction is taken twice daily                   |
| **Rutaceae**         | Lemun tsami                                  | Leaves                                      | Decoction is taken; steam                                                        | Flavonoid, ascorbic acid                         |
| Plant Family | Scientific Name | Common Names | Parts Used | Preparation | Active Ingredients |
|-------------|----------------|--------------|------------|-------------|-------------------|
| Rutaceae    | Citrus aurantifolia | Sour orange | Afofanta (I) Lemu bakagi (N) Orombo wewe (Y) | from the decoction can be inhaled | Vitamin C |
| Rutaceae    | Citrus paradise | Grape fruit | Furuntu (H) Oromo orji (I) Furuntu (N) Lemi iba (Y) | Fruits | Zanthoxyl (antisickling) inulin, alkaloid, vanillic acid |
| Rutaceae    | Zanthoxylum zanthoxyloides | African satin wood | Fasa kwabri (H) Kosonkori (N) Ata (Y) | Root, stem | Decoction is taken as required |
| Solanaceae  | Physalis angulata | Goose berry | Matsar mama (H) Putu (L) Alasagi (N) Koropo (Y) | Whole plant | Decoction is taken as needed |
| Sterculiaceae | Sterculia setigera | - | Kukuki (H) Eso funfun (Y) Kokongiga (N) | Stem bark, seed | Decoction is taken thrice daily with hot water |
| Sterculiaceae | Theobroma cacao | Cocoa tree | Cigban koko (N) | Seed | Decoction of dry seed is taken |
| Sterculiaceae | Waltheria indica | - | Harkufa (H) Arkufe (N) Ewerepo (Y) | Whole plant | Cold water infusion or decoction is taken daily |
| Ulmaceae    | Trema orientalis | Charcoal tree | - | Stem bark, leaf, twig | Decoction or infusion taken orally |
| Zingiberaceae | Curcuma longa | Tumeric | Turi (N) | Rhizomes, fruits | Decoction is taken with milk or sugar |
| Zingiberaceae | Zingiber officinale | Ginger | Tsita maiyatsi (H) Tsutafu (N) Atale (Y) | Leaves, rhizomes | Rhizome is chewed raw; leaf decoction is taken thrice daily |
| Verbenaceae | Lantana camara | - | - | Fruits, leaves | Make tea with either fruit or leaves and take |

**Notes:**
- Syn: Synonym
- D-: D-configuration
- Toxic: Toxic compound

**References:**
- Theobroma cacao: Theobromine
- Zingiber officinale: Gingerol, phellanceren, zingiberene, bisabolene, oleoresin
- Curcuma longa: d-sabinene, cineol, bormeol, zingernie D-phellendrene
- Lantana camara: Lantadene (toxic)
Many plant genera were found to be used either alone or in combination with each other for the treatment of malaria. The plants are mainly of the families Rubiaceae and Apocynaceae and a few from the families Bombaceae, Loganaceae, Rutaceae, Solanaceae, Meliaceae and Gramnieae. Plants used for malaria medicines vary enormously from one community to the other, in both the choice of plant and methods of preparations. Complementary to oral therapy is steam treatment, where the patient is covered with a thick blanket or cloth and subjected to the vapours from a steaming pot of herbs (ingredient of this hot pot include the leaves of lemon grass, paw-paw, mango, and guava). Sometimes the leaves of these plants are used for the preparation of “teas” typical teas are made from lemon grass (Cympogon citratus), lime (Citrus aurantifolia) and sometimes guava leaves. This form of treatment is recommended usually for mild attacks of malaria [16]. Coker and Adesegun compiled a list of over 200 medicinal plants claimed by traditional healers and communities to have antimalarial activity [38].

In spite of several pitfalls encountered in the medicinal plant research, the prospects of developing indigenous drugs for health care delivery systems should be viewed positively [39,40]. In a broader perspective, plants are economic source of a number of important drugs such as morphine, atropine and digoxin. The perpetual biodiversity in nature will continue to provide newer species of plants for production of newer drugs. However, species of plants yielding better quantity of the desired chemicals such as active principles can be developed through genetic engineering while their production can be accelerated through tissue culture techniques [39]. The objective of producing affordable, potent and safer drugs from plants can be met to certain extent by promoting formulations of medicines in their natural or semi-processed form (powder or extracts) from plants as used in traditional medicine for some disorders. Standardization especially in respect of dosages will be necessary through controlled clinical trials to prove their efficacy and safety [39]. Research on medicinal plants need to be conducted across all geographical regions of the world, this will highlight the effect of climate, soil chemistry and other environmental factors on the quality of the plant products [41,42]. The Nupe ethnic group from Bida emirate have been using Abrus precatorius leaf for the treatment of both acute and chronic malarial symptoms. The use of Abrus leaf in the treatment of malaria among Nupes is sometimes either the last option after drugs have failed or due to poverty [43]. High cost, increased toxicity level and emergence of parasites resistant to antimalarial drugs (e.g. chloroquine) have made eradication of malaria by chemotherapy difficult and threatened to render current antimalarials obsolete [44].

3. HISTORICAL BACKGROUND OF MALARIA

Human malaria has been recognized since the earliest period, and the occurrence of mosquitoes trapped in amber suggests its prevalence in prehistoric times [45]. Malaria is an ancient disease recognized by Hippocrates about 400BC. He described the three characteristic stages of malaria attack as chills, high fever and profuse sweating [46]. The first evidence that *plasmodium* was the aetiologic agent of malaria was recognized by Charles Laveran in 1890 as he scanned a live mount of a febrile soldier’s blood at Constantine University Hospital in Algeria [47]. Laveran noted pigment granule containing red blood cells in various forms of elongated or crescents shape disfigurement. Translucent round cells with pigmented granules were also seen, and most notably, amoeboïd-like cells possess long whipping strands that dramatically interacted with and were capable of “drawing in” neighbouring red blood cells. Laveran understood that he was looking at the malaria pathogen and this observation stands as a singular historical event in providing support for a protozoan basis of disease. Twenty years later, MacCallum [48,49] described
exflagellation the whipping motions of the sinuous flagella as the extrusion of male gametes after emergence from within red blood cells a process, which occurred in the gut of the mosquito vector [50,51]. A variety of names were used to describe the disease and they include the shakes, march, noman, jungle, intermittent fever, ague and chills [52]. The work of Laveran, Ross, MacCallum [47,52], and some other malariologists [52] showed the occurrence of developmental cycle in the blood corpuscles and the transmission through mosquitoes. By the early part of the last century, it was believed generally that the broad outlines of the life cycle was known fully, with sporozoites injected by a mosquito bite, thought to enter the cells directly and undergo schizogony. However the actual penetration of a corpuscle was described by Schaudin [46] who observed that sporozoites, on entering the blood did not directly enter red blood cells as formerly thought but within half an hour were carried to reticuloendothelial system (usually, the liver) where they underwent a schizogony circle [45].

4. MALARIAL GLOBAL SITUATION

According to World Health Organization (WHO), each year 300 to 500 million people living in the tropics and subtropics are infected with malaria parasites with nearly 3 million (mostly children) dying [53]. About 1.5 billion people are known to live in the regions where malaria is endemic. The regions are central and northern South America, tropical Africa, North Africa including the Nile valley, parts of Middle East, Central Indian Subcontinents and South East Asia excluding Hong Kong and Macao and East Indies. Malaria is imported into the United Kingdom with 1500–2000 cases reported each year, and 10–20 death. Approximately three-quarters of reported malaria cases in the UK are caused by plasmodium falciparum [54]. About 93% of the 550 million people living in Africa are at risk of malaria and over 90% of the 300-500 million clinical cases reported from Africa [55]. Malaria occurs in 100 countries with about 40% of the world’s population at risk [56]. Malaria epidemics are on the increase due to fabricated conflicts and climate associated disasters causing the movement of non-immune populations to malaria endemic areas [57]. Urban and periurban malaria are now substantial problems in certain areas of Asia and Africa. Malaria is becoming more difficult to manage because of multi-drug resistance [58]. Malaria is directly responsible for one in five childhood deaths in Africa and its resurgence in Africa contrasts dramatically with the global decline in mortality since 1900 [13]. Nigeria accounts for a quarter of all malarial cases in the WHO African region. Transmission in the south occurs all-year round and is more seasonal in the north [12]. In Nigeria malaria affects more people than it did in the 1960s [59]. Fifty percent (50%) of Nigerian population experience at least one episode of malaria every year. The Federal Ministry of Health reported that one in four people suffer from malaria at one time or the other [2]. Transmission in tropical countries is highly heterogenous spatially and seasonally. Plasmodium falciparum is known to cause the majority of severe clinical disease [60].

About 110 million Nigerians are at risk of infection from malaria parasites [61] and malaria is responsible for deaths before the age of 5 years in 1/5 and 1/3 of children in urban and rural areas respectively [62]. In Nigeria and probably and other parts of West Africa, the predominant parasite is Plasmodium falciparum (75%). Others are Plasmodium malariae (15%) and Plasmodium ovale (3%) with Plasmodium vivax probably not found in West Africa [63]. Funding for malaria control in Nigeria was increased from US$ 17 million in 2005 to US$ 60 million in 2007, provided by the government, the Global Fund and the World Bank [12]. False positivity under reporting of mixed infections and a significant number of species mismatch needs attention and should be improved for appropriate diagnosis. The detection of substantial of false positive results by molecular methodologies may provide the accurate
incidence of circulating plasmodium species in the geographical regions and has important repercussions in understanding malaria epidemiology and subsequent control [64]. More systemic, timely, and empirically based approaches are urgently needed to track the rapidly evolving landscape of malaria transmission in Africa [65].

5. CLASSIFICATION OF MALARIA

Malaria fever has been categorized as benign, simple or tertian (caused by *Plasmodium vivax*) or aestivo-autumnal, malignant tertian, pernicious quotidian, subtertian or tropical (caused by *Plasmodium falciparum*) or quartan ague or quartan malaria (caused by *Plasmodium malariae*) or ovale tertian malaria (caused by *Plasmodium ovale*). *Plasmodium vivax* shows the widest distribution, being prevalent throughout the tropics and many temperate regions and characterized by relapses: reappearances of symptoms after a latent period of up to 5 years, as is infection with *Plasmodium ovale*, which occurs chiefly in tropical Africa. Such relapses are due to the sudden activation of hypnozoites (sleeping merozoites) in liver cells. *Plasmodium malariae* is much less common than *Plasmodium vivax* and *Plasmodium falciparum*. Although falciparum malaria and malariae malaria do not show relapses, they are subjected to ‘recrudescence’ repeated manifestation of infection after a relatively short latent period between 3 months and 1 year [45]. Malaria genetics reveal many peculiarities. There is immunity amongst Africans living in endemic areas that are exposed to repeated reinfection (premonition). There are racial differences in susceptibility; and resistance is associated with certain genetic factors; e.g. haemoglobin S, which is common amongst Africans with sickle cell anaemia trait with lethal effect [2].

6. BIOLOGY OF MALARIA PARASITES

The plasmodium life cycle is complex with a number of different forms that differ in microscopic appearance and antigenicity. In the human host, sporozoites, the infectious form injected by the mosquito, are carried by the blood stream to the liver, where they infect liver cells. In these cells, each parasite enlarges and subdivides, producing thousands of merozoites, which are then released into the bloodstream and establish the cycle involving the erythrocytes. The parasite grows and divides in the erythrocytes of the host. The earliest form resembles a ring, with a large pole food vacuole in the central area, with the nucleus and cytoplasm being pushed to the periphery. This develops into a larger motile trophozoites, which goes on to subdivide, producing a schizont. The infected erythrocytes then break open, and the offspring of the division called merozoites (6–32) which after 5–16 days, months or years [66] are released into the plasma. The duration of this cycle varies with species of plasmodium, *P. falciparum* (6 days), *P. ovale* (9 days), *P. vivax* (8 days) and *P. malariae* (14 days). The merozoites then enter new erythrocytes and multiply, repeating the cycle. The erythrocytic cycle takes 2 days for *P. falciparum*, *P. vivax* and *P. ovale* and 3 days for *P. malariae*. The attack of fever occurs at the rupture of the erythrocytes and release of the merozoites [62]. The symptoms last for 12 to 24 hours [67]. Some merozoites that enter erythrocytes develop into gametocytes, which are specialized sexual forms. Male and female gametocytes transforms into about half dozen tiny whip-like gametes that swim about until they unite with the female gamete. The resulting zygote transforms into a motile form that burrows into the wall of the midgut of the mosquito and forms a cyst, which enlarges as diploid nucleus that undergoes meiosis, dividing asexually into numerous offsprings. The cyst then ruptures into the body cavity of the mosquito, and the released sporozoites find their way to the mosquito’s salivary gland and saliva, from which they must be injected into a new human host [68,69]. The incubation period for malaria is between 10–
35 days [66]. Mosquito is the definitive host. The only function of human is to enable the parasites infect more mosquitoes, so that further sexual recombination can occur. Asexual cycle takes place in man while sexual cycle takes place in mosquito [70]. Although malaria can be transmitted by transfusion of infected blood, congenitally, and by sharing needles, infection usually is transmitted by the bite of infected female Anopheline mosquitoes. Once the tissue schizonts burst in *P. falciparum* and *P. malariae* infections, no forms of the parasite remain in the liver. High prevalence of placental malaria has been reported in Nnewi, South Eastern Nigeria [71]. However, in *P. vivax* and *P. ovale* infections, tissue parasites (hypnozoites) persist and can produce relapses of erythrocytic infection months to years after the primary attack. Once plasmodia enter the erythrocytic cycle, they cannot reinvade the liver; thus, there is not tissue stage of infection for malaria contracted by transfusion. The merozoites invade more erythrocytes to continue the cycle, which proceeds until death of the host or modulation by drugs or acquired partial immunity. For erythrocyte invasion, merozoites bind to specific ligands on the red cell surface [70]. *P. falciparum* has a family of binding proteins that can recognize a number of host cell molecules, including glycophorins A, B, and C, as well as band 3. It is able to invade all stages of erythrocytes and therefore can achieve high parasitemias, *P. vivax* is more selective in its binding; it needs to recognize the Duffy chemokine receptor protein as well as reticulocyte-specific proteins; thus, it will not establish infection in Duffy-negative individuals and will only invade reticulocytes. Because of this restricted subpopulation of suitable erythrocytes, *P. vivax* rarely exceeds 1% parasitaemia in the bloodstream. But in Asia and the America, *P. vivax* is a more common cause of malaria [72]. The Fy(a-b-) phenotype is most common in the area where there is little *P. vivax* malaria [73]. *P. ovale* is similar to *P. vivax* in its predilection for young red blood cells, but the mechanism of its erythrocyte recognition is unknown. *P. malariae* recognizes only senescent red cells, maintains a very low parasitemia, and typically cause an indolent infection [74]. *P. falciparum* assembles cytoadherence proteins (the PIEMPs encoded by a highly variable family of var genes) into structures called knobs on the erythrocyte surface. This allows the parasitized erythrocyte to bind to the vascular endothelium, to avoid the spleen, and to grow in a lower oxygen environment. For the patient, the consequences are microvascular blockage in the brain and organ beds and local release of cytokines and direct vascular mediators such as nitric oxide, leading to cerebral malaria [75]. Recently, plasmodium cynomolgi a monkey plasmodium species has been discovered in a 39-year-old woman from malaria free area with no previous history of malaria or travel to endemic area [76].

7. PATHOGENESIS OF MALARIA

*Plasmodium falciparum* is not only the most common in Africa but also, is the most virulent, and enjoys the reputation as the greatest killer of mankind being particularly dangerous to children [2] and responsible for all severe complications and deaths [3]. Infective female Anopheles mosquito injects saliva containing plasmodia sporozoites, which enter the parenchyma cells of the liver usually within 1 hour [1]. The merozoites rupture out from the liver into blood stream and invade erythrocytes [77]. Each species has a specific receptor on erythrocytes it attaches. For example *Plasmodium vivax* attaches to the duffy blood group antigen and many natives of West Africa lack this antigen and therefore are resistant to *Plasmodium vivax* [78]. Genetic resistance to malaria occurs through both modifications of the immune system that enhance immunity to this infection and also by dangers in human red blood cells that hinder the malaria parasite’s ability to invade and replicate within these cells. Host resistance involves blood cell genes and abnormal hemoglobins [79]. Several inherited variants in erythrocytes have become common in formerly malarious parts of the world as a result of selection exerted by this parasite [81]. Persons with α- thalassemia HBC
and HBE have some degree protection against the parasite [82]. South-East Asian ovalocytosis offers protection against cerebral malaria in children by reducing sequestration of erythrocytes parasitized by *P. falciparum* in the brain microvasculature [82]. Adhesion of *P. falciparum*-infected red blood cells to CD36 enhanced by the trait and higher efficiency of sequestration could determine a different organ of distribution of sequestered infected red bloods cells [83]. Endogamy along caste and ethnic lines appear to have confined malaria resistance via multiple genes to many community of Nepal and India [84]. Humoral and cell-mediated immune response limit malaria parasite multiplication and many cytokines contribute to pathogenesis and resolution of malaria [85]. The infections in the millions of different red blood cells nearly become synchronous, rupture and release daughter protozoa at the same time. For *P. malariae* the cycle takes 72 hours so that fever appears every third day [69]. In other species of plasmodia, rupture occurs every 48 hour. The liberated merozoites rapidly infect a new population of erythrocytes initiating the next cycle of fever and chills [78]. The merozoites enter red cells and differentiate into male and female gametocytes, which are ingested by blood sucking female Anopheles mosquito. *Plasmodium vivax, Plasmodium malariae* and *P. ovale* parasitaemia are of relatively low grade because the parasites favour either young or old red cells; *Plasmodium falciparum* invades red cells of all ages including the erythropoietic stem cells in bone marrow, so parasitaemia may be very high [77]. *Plasmodium vivax* and *Plasmodium ovale* prefer young red cells and *Plasmodium malariae* prefer aged red cells. *Plasmodium falciparum* has no secondary phase and the infection is resolved in 3 years (usually within 1 year) [79]. *Plasmodium malariae* had occurred as long as 10-53 years in the infected humans after initial exposure especially after splenectomy. The spleen appears to be involved in the prevention of maturation of parasites thereby preventing reinfection [78]. *Plasmodium malariae* with its prolonged latency is the most common cause of transfusion malaria in non-endemic areas, whereas *Plasmodium falciparum* and *Plasmodium vivax* are the most common cause of malaria worldwide [74]. Patients with malaria often exhibit laboratory abnormalities due to an acute phase response. Lipid profile changes are characteristic for malaria [86]. Changes in high-density lipoprotein and very low-density lipoprotein in human serum are related to lipid metabolism of *Plasmodium vivax* [87]. Dialated cardiomyopathy related cytomegalorirus–induced myocarditis has been reported in vivax malaria in an Amazonian child [88].

8. PATHOLOGY OF MALARIA

The most characteristic symptom of malaria is the occurrence of paroxysm of fever with temperatures of up to 40-41°C at regular intervals—every 48 hours (*Plasmodium falciparum, Plasmodium ovale* and *Plasmodium vivax*) or 72 hours (*Plasmodium malariae*) tertian or quartern fever—alternating with good periods of no fever [2,89]. The periodicity of parasitaemia and febrile manifestation is preceded by headache, lassitude, loss of appetite, muscle pain and chills, resulting in violent uncontrollable shivering with teeth chattering, accompanied by thirst, nausea, vomiting and in severe cases sometimes by delirium and convulsion in children frequently ending in death. These signs are more pronounced usually in falciparum malaria [2]. There is temporal lagged association between meteorological factors and malaria which depend on the climatic condition. Therefore, the lag pattern for meteorological factors should be considered in the development of malaria early warning system [90]. Malaria was perceived as the world’s worst health problem [91]. More people are dying each year from malaria than 30 years ago and malaria is returning to areas from where they had been eradicated. High level of malaria parasitaemia has been observed in African children with symptomatic HIV infection. These children have been found to be protected against cerebral malaria and invariably deaths due to cerebral malaria. This has been attributed to lower levels of tumor necrosis factor, which boost antibodies against
malaria in HIV infected children [92-94]. However, concurrent infections with *P. falciparum* and *W. bancrofti* or *M. perstans* has been reported in children and young adults in Mali [95]. Hemozoin-dependent induction of matrix metalloproteinases-g (MMP-9) expression and activity has been demonstrated in mononuclear and endothelial cells [96] and so MMPs should be taken into account as potential new targets for an innovative and adjunctive therapy for severe malaria [97].

Malnutrition, splenomegaly and anaemia are the expected complications of repeated attacks of malaria. However, acute falciparum malaria produces complications collectively known as pernicious or malignant malaria. This arises from interruption of blood flow in the capillaries that interferes with oxygen supply causing anoxia and may eventually lead to rupture of the capillaries and bleeding into the tissues, invariably resulting in death [1,2]. There are two main types of pernicious malaria: cerebral malaria, which causes brain damage resulting from the clogging of the brain capillaries with symptoms as severe headache, convulsions, delirium and other psychotic signs with temperature rising up to 42°C. Cerebral malaria frequently ends in death and is the most lethal of all the malaria. The other is agid malaria, involving other organs of the viscera: the signs in the patients are cold clammy skin, with high internal temperature as well as severe vomiting, diarrhea and sweating. The consequences are never as severe as in cerebral malaria, but extreme exhaustion may lead to prostration and unconsciousness [2]. In Africa, 30 million women living in malaria-endemic area become pregnant each year, with up to 200,000 new born deaths each year as a result of malaria in pregnancy [98].

9. DIAGNOSIS OF MALARIA

Malaria is a disease of many facets with symptoms that trigger most of the diseases of man [7]. Jeffery divides the parasitaemia of man into four phases: preclinical, clinical, terminal (asymptomatic), and relapse (asymptomatic and symptomatic). [99] Bray designated another prior to the preclinical as the pre-erythrocyte phase, which lasts only 24 hours, as a period of cytoplasm fission of a multinucleate mass. Preclinical period follows the appearance of the parasite in the peripheral blood and which is the time when frank manifestations appear. [100] Periods of chill alternating with longer febrile intervals are the classical manifestations [8,101].

Malaria parasites in blood sample stored at 4°C are viable for at least a period of one week. *Plasmodium falciparum* can remain viable for a period of 2 weeks [7,74]. A definite diagnosis of malaria infection is established on the finding of parasites in the blood. Malaria should be suspected in all cases of fever in endemic areas or in persons who have been exposed to the infection. In drug resistant core endemic region, anti-inflammatory effect on white blood cells is demonstrated using a simple: economic monocular microscopic that can be used in day light or with aid of lamp [102]. Microscopically diagnosis is only as reliable as the competence of the workers who prepare the blood slides and examine such slides. Diagnosis of human malaria is mainly by the detection of plasmodium species from microscopic examination of the blood. One should remember that the presence of malaria parasites in the blood is a sign of infection but not necessarily a cause of the disease [56,61]. Although various modern methods ranging from density high-speed centrifugation, with monoclonal antibodies to the application of magnetic separation techniques, DNA probes and even the newer amplification technique such as the polymerase chain reaction (PCR) have been tried, in order to detect scanty parasitaemias. It appears that the time honoured thin-and thick film blood examination by a competent microscopist remains unchallenged when it comes to simplicity and convenience [61]. But the flow cytometry
method is a simple robust, and efficient method for detecting \textit{P. vivax}–infected reticulocytes [103]. But PET-PCR, a new molecular diagnostic tool with similar performance characteristics as commonly used PCR methods that is less expensive, easy to use and amiable to large scale–surveillance studies in developing country settings [104]. The pLDH antigen in three RDTs, used in combination with HRP2 provides added diagnostic specifically of malaria parasitaemia and may be useful to distinguish acute infection from recently treated infections where diagnostic specifically is desirable (e.g. for selection of malaria infected participants in clinical trials) a three-band RDT should be considered in sub-Saharan African [105].

Malaria rapid diagnostic tests (RDTs) are qualitative immune-chromatographic lateral flow tests in dipstick (strip), cassette or card form that detect malaria antigen in peripheral blood. Malaria antigen from a lyzed blood sample is reacted with anti-malarial monoclonal antibody conjugated to colloidal gold (pink-mauve) particles. The antigen-antibody colloidal gold complex migrates along the nitrocellulose membrane where it becomes bound (capture) by a line of specific monoclonal antibody, producing a pink line in the test result area. The line can be seen after a washing buffer has removed the background haemoglobin. A further pink line, i.e. inbuilt positive control, is produced above the test line indicating that the test reagents have migrated satisfactorily (it is not a malaria antigen control) [56].

Radioimmunoassays have also been tried for malaria diagnosis. Antibodies to malaria can be detected using enzymatic immunoassays or immune-fluorescence techniques. The antibodies to the asexual blood stages appear days to weeks after the infection and may persist for months. Although useful in survey work or for screening blood donors and reducing wastage, they are of little value in the “acute” malarial situation [61].

Solid-phase inhibition radioimmunoassays have been used to demonstrate parasite antigens. These assays use solubilized erythrocytes infected with \textit{P. falciparum} and are based on the ability of washed infected red blood cells to inhibit the binding capability of radio-or enzyme-labelled antibody on a plastic of microtitre plate precoated with crude extracts of malaria antigen obtained from \textit{in-vitro} cultures of \textit{P. falciparum}. Such test systems are useful where low parasitaemias in the range 5-50 asexual parasites/\textmu l of blood are found. An inhibition radioimmunoassay test based on a monoclonal antibody labeled with a radioisotope, iodine -125, and used in an antibody ‘sandwich, has also been described. In field trials it produced a detection level of >1 asexual parasites of \textit{P. falciparum}/ \textmu l of blood; better than one would expect from routine light microscopy [61].

Serological methods of diagnosis of malaria have become of practical value since 1962 when the indirect fluorescent antibody test (IFAT) was introduced. In the IFAT procedure the antigen consists of a film of infected blood on a microscope slide. The slide is covered first with one of the serial dilutions of the test serum; then it receives a solution of antihuman globulin labeled with fluorescein isothiocyanate; after washing and drying, the slides are examined in a fluorescence microscope. Antibody in the test serum reacts with antigen of the malaria parasites and the antiglobulin reaction with the antibody is indicated by the fluorescence of the parasites. Fluorescence of the last serial dilution is given as a ‘titre’ of the antibody present [61].

In recent years a number of new techniques based on the “dipstick” format, have become available for the diagnosis of malaria. These include the ICT-Malaria PF, OptiMALr and the Kat-Quick kits. The methods are based on the principle of the detection on plasmoidal histidine rich protein-2 (HRP-2) or parasite-specificities lactate dehydrogenase (PLDH) which
is present in *P. falciparum* and other Plasmodial infections. A number of reports claim sensitivities and specificities approaching 100% while other reports have claimed up to 6% cross reactivity with sera positive for rheumotoid factor. Some of these “dipstick” methods have been extended to include screening for other forms of malaria but to date results have not been quite impressive [56]. The performance of HRP-2 test in detecting asymptomatic carriers of *P. falciparum* varies by age and parasite density. Its low sensitivity may limit its utility in pre-elimination interventional settings. So loop-mediated isothermal amplification in combination with cost effective HRP-2 test may be worth exploring in such setting [106]. But Luciferase-expressing *plasmodium berghei* parasites to measure pre-patent period of malaria infection in rodents using a bioluminescence assay is novel, simple, fast and sensitive. The sensitivity and accuracy of this new method is comparable to standard PCR and microscopy-based techniques respectively [107].

Dipstick tests have the potential of enhancing the speed and also the accuracy of diagnosing *P. falciparum*, particularly in non specialized laboratories where inexperienced or junior staff may be involved, since very little training is required for these techniques. A potential problem with these methods is that the circulating antigen may be detected for many days up to 2 weeks in a laboratory after the elimination of viable parasites from the circulation. It must therefore be remembered that a positive test may not always be due to an active infection. The dipstick methods should be regarded as useful additional tests to the long established diagnostic method of examining thick and thin blood films regarded as the standard, not as replacement methods [56].

Molecular biological detection tests have also been used for diagnosis and are known to use DNA and RNA probes. DNA probe is based on principle of hybridization with two complementary strands of the DNA helix separated (denatured) by chemical means, or heat treatment. The separated strands of DNA are then put into contact with a DNA probe. This probe has been obtained using recombinanat DNA techniques, or produced synthetically. Most of the available DNA probes are specific to *P. falciparum* and sensitivities as low as 5 asexual parasites/µl of blood have been reported, but unfortunately, these results have only so far been obtained with radioisotopic method using iodine 125 or phosphorus 32. Enzyme-based systems with biotin and others are rather less sensitive although new probes specifically designed for enzymes detection may improve these levels considerably [56].

Ribonucleic acid (RNA) probes have also been evaluated with promising results. Unfortunately, RNA is less stable and cross-species reactivity could occur. RNA probes have been developed for all four human malarias and show an increased sensitivity due to the high number of target sequences which occur in RNA. Detection techniques using 12-hour autoradiography exposure have reportedly produced detection levels as low as few parasites/µl of blood. No effective non-radioactive label has yet been reported [61].

Other methods of diagnosis include the QBC II, Becton-Dickinson’s Quantitative Buffy Coat (QBC) method. This involves centrifuging the patient’s blood in special capillary tubes precoated with Acridine Orange (AO) in which parasite DNA is stained with AO. A small precision moulded plastic float presses the parasitized red cells (which occupy the upper most part of the red column) against the wall of the tube, where they can be viewed by ultra violet light microscopy. The sensitivity of this method is claimed to be very high with experienced users, although some reports suggest that young trophozoites of *P. falciparum* and *P. vivax*, could not be distinguished with any degree of certainty and that confirmatory blood films should always be examined. Additionally special equipment is required, which may preclude the method from being used in smaller centres [61].
Another relatively new method is the polymerase chain reaction (PCR) which uses a non-isotopically labeled probe following PCR amplification. It is possible to detect less than 10 parasites in over 10µl of blood. PCR may yet prove to be a valuable addition to the examination of blood films for the diagnosis and speciation of malaria. Again, the special equipment required precludes all but the larger centre. Some researchers have claimed that PCR (and ELISA) techniques are as sensitive as blood films, however they are infinitely more expensive, require specialized equipment and take a longer time to complete [56]. The RDTs detected *plasmodium* in *P. knowlesi*-infected blood samples has poor sensitivity and specificity. Patients with *P. knowlesi* could be misdiagnosed as *P. falciparum* with OptiMal-TT, *P. vivax* with paramax-3 and more correctly as non-*P. vivax* non-*P. falciparum* with Binax Now® malaria. Therefore, there is a need for a sensitive and specific RDT for malaria diagnosis in settings where *P. knowlesi* infections predominate [108].

10. TREATMENT OF MALARIA

Malaria is the classical example of a disease that affects the productivity of individuals, families and the entire world due to morbidity and mortality [109]. A key component of any transmission reduction strategy must be methods to attack the parasite as it passes from man to mosquito (and vice versa). The understanding of such methods should be rationally based on molecular, cellular, population to the evolutionary levels [110]. But no single intervention will significantly lower the burden of imported malaria [111]. The first antimalarial drug was quinine isolated from the bark of cinchona specie (Rubiaceae) in 1820. In 1940, chloroquine was synthesized and until recently was the only drug used for the treatment of malaria. Chloroquine resistant *P. falciparum* is treated with alternative drugs or drugs combinations, which are expensive and sometimes toxic [112]. Chloroquine and hydroxychloroquine have been proven to have antiretroviral activity both *in-vivo* and *in-vitro* hence could be used to treat co-infection of malaria and HIV [113]. Mechanism of action of chloroquine remains controversial, the agent probably acts by concentrating in parasite food vacuoles, preventing the polymerization of the haemoglobin break down product, haemozoin and thus eliciting parasite toxicity due to the abundance of free haem. Resistance in *Plasmodium falciparum* has been attributed to mutations in a putative transporter PFCRT, however the clinical value of resistance-reversing drugs is not established. Chloroquine remains the drug of choice for the treatment of sensitive *P. falciparum* and other species of human malaria parasites. It rapidly terminates fever (in 24-48 hours) and clears parasitaemia (in 48-72 hours) caused by sensitive parasites. It is safe, cheap and many partially immuned individuals respond to chloroquine treatment [3]. Artemisinin and its derivatives are effective against drug-resistant *Plasmodium falciparum* and so they are of utmost important in the current antimalarial campaign [109] Artemisinin or Qinghaosu is a sesquiterpene lactone endoperoxide isolated from Chinese herb Artemisia annua L. (Asteraceae) that has been in use in traditional treatment for chills and fevers for more than 2000 years [114]. Artemisinin was isolated pure in 1972 and its structure was determined in 1979 [115]. Quick reduction of fevers, fast clearing of parasites from blood (90% of malaria patients recovered within 48 hours) are characteristics of artemisinin. WHO recommended that all countries experiencing resistance to conventional monotherapies should use combinations containing artemisinin derivative (artesunate) [112] and other agents such as amodiaquine and fansidar [116]. Lapdap that contains chloroguanol hydrochloride and dapsone is one of the safest antimalarials in use [117]. Terkuile *et al.* strongly recommended household level treatment for presumed malaria [118].

Fansidar® may be used against chloroquine-resistant and presumptive falciparum malaria. Intermittent preventive treatment during the pregnancy with optimal doses of sulphadoxine-
pyrimethamine protects pregnant women from malaria related adverse outcomes [119]. A number of antibiotics in addition to the folate antagonists and sulphonamides are modestly active antimalarials but their mechanisms of action are unclear [3,118]. The sulphadoxin/pyrimethamine combination had been used to treat uncomplicated falciparum malaria for more than 30 years. Non-silent mutations in dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) genes are responsible for the resistance to pyrimethamine and sulphadoxine respectively [87]. The discovery of techniques for continuous maintenance of human *Plasmodium falciparum* *in vitro* has led to practical assays of susceptibility of these organisms to antimalarial drugs [120]. The biology of malaria infection must be appreciated for understanding of action and therapeutic uses of antimalarial drugs [44].

Malaria is the most important of the transmissible diseases. The incubation period of malaria is 10-35 days and since there are no effective drugs against sporozoites, infection with the malaria parasite cannot be prevented. Sporozoites develop into merozoites after 5-16 days and after months or years are released from liver into circulation. But *Plasmodium falciparum* has persistent hepatic circle. Primaquine, proguanil and tetracyclines (tissue schizonticides) act on this site and are used for radical cure. Chloroquine, quinine, mefloquine, halofantrine, proguanil, pyrimethamine and tetracyclines kill these asexual forms. These drugs are used to treat and prevent acute attacks called suppressive prophylaxis. Quinine, mefloquine, chloroquine, artesunate, arteether and primaquine (gametocides) prevent the transmission of infection [62]. But artemisinin combination therapy (ACT) is currently being used as the first line therapy for falciparum malaria [87]. With improvements in surveillance systems linked to improved diagnosis of malaria. The use of satellite imagery and mobile phone have complimentary and contemporarybenefits in infectious disease control and elimination [121].

### 11. CLASSIFICATION OF DRUG TYPES USED IN TREATMENT OF MALARIA

The drugs which are effective in the treatment of malaria may be divided into two groups; drugs which act on the asexual stage of the malarial parasite in the blood—quinine, chloroquine, proguanil, halofantrine, mefloquine and pyrimethamine and drugs which act on the exo-erythrocyte stage in the liver and gametocytes—primaquine. Doxycycline is effective against resistant *P. falciparum* and has been used with success in the far East. It should be taken after meals with copious fluids. There are two ways in which malaria can be attacked by drugs—chemosuppressive and treatment of established disease. Suppressive treatment means the regular administration of a drug to prevent the clinical manifestation of the disease. The best drug for this purpose varies in different parts of the world. *P. falciparum* strains from many parts of the world are resistant to one or more antimalarial drug [122].

Drugs for prevention of malaria include chloroquine (500mg weekly), mefloquine (250mg weekly), doxycycline (100mg daily), malarone 1 tablet (250mg atovaquone/100mg proguanil) daily, primaquine 26.3mg (15mg base) daily for 14 days and proguanil (200mg daily) as an alternative to mefloquine. Drugs that eliminate developing or dormant liver forms are called tissue schizonticides (e.g. primaquine and tafenoquine), those that act on erythrocytic parasites are blood schizonticides (e.g. quinine, chloroquine, halofantrine, arteflene, artesunate) and those that kill sexual stages and prevent transmission to mosquitoes are gametocides (e.g. primaquine, chloroquine, quinine). No one available agent can reliably effect a radical cure that is eliminate both hepatic and erythrocytic stages. Few available agents are causal prophylactic drugs that are capable of preventing erythrocytic infection. All effective chemoprophylactic agents kill erythrocytic parasites before they grow sufficiently in numbers to cause clinical disease. Fansidar is commonly used to treat uncomplicated falciparum malaria [3]. Combination drug therapy, administered concomitantly or consecutively, is common with antimalarial drugs [123]. Proguanil, pyrimethamine,
sulphadoxine and dapsone are antimetabolites whereas tetracycline, doxycycline and minocycline are antibiotics [65]. Causal prophylactics (e.g. proguanil, primaquine) attack pre-erythrocytic phase; suppressive prophylactics (e.g. chloroquine, mefloquine) suppress the erythrocytic phase; suppressive cures are a form of radical cure by extended suppressive therapy (e.g. chloroquine 300mg weekly for 10 days). It may involve slow action on hypnozoites. Clinical cure involves the use of erythrocytic shizonticides (e.g. atovaquone, artemisinine, halofantrine) to terminate episode of malarial fever. But radical cure involves the use of drugs (e.g. primaquine 15mg daily for 2 weeks) to attack the hypnozoites given together with a clinical curative to achieve total eradication of the parasites [69,124].

The aims of using drugs in relation to malarial infection are: to prevent and treat clinical attack of malaria; to completely eradicate the parasite from the patient's body; and to reduce the human reservoir of infection-cut down transmission to mosquito. These are achieved by attacking the parasite at its various stages of life cycle in the human host. Antimalarial drugs exhibit considerable stage selectivity of action. A single 45mg (0.75mg/kg) dose of primaquine is employed immediately after clinical cure of falciparum malaria to kill the gametes and cut down transmission to mosquito [123].

12. CHEMICAL CLASSIFICATION OF ANTI-MALARIAL DRUGS

There are several major chemical classes of antimalarial drugs. These include 4-aminoquinoline used for treatment and chemoprophylaxis of infection with sensitive parasites (e.g. chloroquine, hydroxychloroquine, amodiaquine), quinoline methanol (e.g. quinidine, mefloquine and cinchona alkaloid (quinine) used to treat chloroquine resistant P. falciparum, 8–aminoquinoline used for radical cure and terminal prophylaxis of infections with P. vivax and P. ovale (e.g. primaquine, bulaquine), biguanides (folate antagonist) for chemoprophylaxis (e.g. proguanil)[62], tetracyclines for treatment of infections with P. falciparum and chemoprophylaxis (e.g. tetracycline, doxycycline), phenanthrene methanol (e.g. halofantrine) for treatment of infections with some chloroquine-resistant P. falciparum, amyl alcohol (lumefantrine) for treatment of P. falciparum in fixed combination with artemether (coartem)[68], sesquiterpene lactone endoperoxides (artesunate, artemether, arteether) for treatment of infection with multidrug resistant P. falciparum and quinonefolate antagonist combination (e.g. malariae: atovaquone/proguanil) for treatment and chemoprophylaxis of P. falciparum infection [3]. Other classes; are Hydroxynaphthaquine (atovaquone) has activity against plasmodium species, diaminopyrimidines (e.g. pyrimethamine) exhibited potent antimalarial activity. Antimalarial activity of proguanil is ascribed to cycloguanol, a cyclic triazine metabolite and selective inhibitor of the bifunctional plasmodial dihydrofolate reductase thymidylate synthetase, sulphonamides (e.g. sulphadoxine) and sulphones (e.g. dapsone) have antimalarial activity but are slow acting schizonticides that are more active against P. falciparum than P. vivax. As paraaminobenzoate analogues that competitively inhibit the dihydropteroate synthase of P. falciparum. The sulphonamides are used together with an inhibitor of parasite dihydrofolate reductase to enhance their antiplasmoidal action. Dapsone given with the chloroquinil also has been effective for therapy of chloroquine-resistant P. falciparum malaria [65]. Acridine (e.g. mepacrine), anerythrocytic schizonticide, more toxic and less effective than chloroquine [62,123]. The efficacy of artemisinin based combination therapy has been established. Artemether+humefantraine, Artesunate+ sulphamethoxypyrazine-pyrimethamine, Artesunate+Amodiaquine and sulphone–Pyrimethamine+Amodiaquine have almost sme efficacy. Defervescen was taster with Artesunate+Amodiaquine than Artesunate+Lumefantrine [125]. Fatigue was more frequent in patients receiving Amodiaquine than by those treated with Artesunate+ Sulphamethoxypyrazine–pyrimethamine or Artesunate and Lumefantrine [126].
OMARIA the antimalarial said to be effective against *P. falciparum* and *P. vivax* uses ellagic acids and tannins. But alkaloids may have deleterious effects on the placental parenchyma and ellagi-tannins seem to up-regulate healthy conditions and post partum [127]. All these point the fact that workers across the globe are making intent efforts to combat malaria [128,129]. Firstever White House Summit on malaria focuses on life-saving initiatives announced the president’s malaria initiative (PMI), a five year, $ 1.2 billion programme for combating malaria in 15 of the hardest-hit countries in Africa. Aid has already reached 6 million Africans from Angola, Tanzania and Uganda [130].

Chloroquine and mefloquine (Long-acting quinolines) are used for chemotherapy and chemoprophylaxis of malaria, although widespread resistance is now associated with chloroquine while mefloquine treatment is associated with toxicity and resistance development. Quinine a short acting quinoline is effective in the treatment of severe and drug resistance malaria, but has a potent effect on the cardiovascular system [22]. The protective efficacy of tafenoquine (200mg daily for 3 days followed by 200mg weekly maintenance doses is similar to that of weekly standard of care (methoquine, 250mg) [131].

Primaquine, a quinoline active against hepatic phase of some malaria parasites is contraindicated in glucose 6-phosphate dehydrogenase deficiency and pregnancy [124]. Atovaquone, which is given in combination with proguanil, is not effective for *P. vivax* malaria, so it is not used for children under 11kg, pregnant women or those breast-feeding, and is contraindicated in patients with severe renal impairment [110]. *P. vivax* malaria can no longer be considered a benign condition. WHO guide line for treatment of *P. vivax* need to be reinforced [132]. Diaminopyrimidines used in combination with sulphad drugs (e.g. pyrimethamine-sulphadoxine) in the treatment of chloroquine resistant malaria, however, it is known that sulphonamides are potentially toxic and resistance to the antifolates is prevalent [133]. Sesquiterpene lactone endoperoxide derivatives such as artemisinin, dihydroartemisinin, artemether and artesunate obtained from *Artemisia annua* a Chinese plant although has been in use for more than three decades, no report of systemic human toxicity study on the plant. However, in the pre-clinical toxicity study, lesions occurred in the brain, liver, bone marrow and fetus [23]. Findings revealed that about 95.4% of the plant showed in vitro antiplasmodial activities are from African and Asian continents. But only 2.9% of the plants were studied *In vivo*. However, 19.5% of the plants showed promising in vitro antiplasmodial activities (IC$_{50}$>5.0µg/ml). However one in every four plants showed promising *in vivo* antiplasmodial activities (cleared at 25–50mg/kg between 50–99% parasites from the blood in 4 days). There is no correlation between in vitro and in vivo antiplasmodial activities of medicinal plant extracts and phytochemical principles [134]. Sulphadoxine-pyrimethamine remains effective at clearing existing infections when provided as intermittent preventive therapy in pregnancy [135].

### 13. *In vitro* ANTIPLASMODIAL ASSAY

The testing of new anti-malarial drugs requires that at least two steps are undertaken before testing in human may take place. In the first step, the new drug is tested *In vitro* and then-if promising results are obtained—*In vivo* [136].

In the first step of process, assays have been developed that focus on the drug’s ability to affect parasite growth in red cells. In these assays, *P. falciparum* is the parasite used if the drug is intended for humans [136]. *P. falciparum* drug sensitivity assays are performed by monitoring the accumulation of the parasite protein, Histidin–Rich protein 2. (HRP 2) in the culture after lysis of the parasite cells. Quantification of HRP2 is assessed by a double-site
antigen capture ELISA as described by [105]. The photometric reading obtained and the corresponding log drug concentrations are fitted to a dose response curve model using an automated curve-fitting analytical software (Table curve 2D version 4). The results are expressed as the drug concentration resulting in 50%, 90% and 99% inhibition of parasite growth (IC\(_{50}\), IC\(_{90}\), IC\(_{99}\)) [137]. The development of a continuous in vitro culture system for \(P. falciparum\) by Trager and Jensen has provided an extremely useful system for analysis of the effects of drugs on \(P. falciparum\) [145]. But several constraints limit the use of this system for drug screening [138].

Modification of existing continuous \textit{In vitro} culture methods for \(P. falciparum\) involves the maintenance of the parasite in RPMI/640 medium supplemented with human or rabbit serum or with the H-hypoxanthine supplemented bovine serum. The antiparasite effect of the test drug can be screened routinely against \(P. falciparum\) growth in bovine serum supplemented with H-hypoxanthine. Drug effect may be rapidly and accurately determined by monitoring the incorporation of H-hypoxanthine into parasite nucleic acids [138]. OMARIA is highly potent against all field isolates. Lethal dose on vero cells indicate a selective index of 13 for \(P. falciparum\) strains FCB. Hence OMARIA can be used in searching African phyto parables for use in Africa with similar results as in India and in new drug design [139].

Another modification of \textit{In vitro} assay is green fluorescent protein (GFP) system in which the \(P. falciparum\) protein binds to GFP, which produces a fluorescence that can normally be detected by a microplate reader. However, fluorescence activated cell sorter (FACS) has been used to assess the parasite growth. Also, GFP-fluorescence needs to be performed in live parasites, besides the fact that ring-form stages of the parasite containing GFP are difficult to distinguish from unaffected cells, which are estimates. Therefore, to be able to quantify parasite growth, luciferase protein can be used in place of microplate reader. Luciferase reporter protein system is used. The luciferase protein is also the result of light-emitting reaction. To build a DNA construct, the Luciferase gene is strategically engineered next to a specific DNA sequence known as a promoter, which is a gene-specific sequence that triggers transcription. The luciferase system is used to investigate whether a promoter of interest is working. The working promoter transcribes the luciferase gene that results in the production of the luciferase protein which then emits light that can be quantified using a microplater. But to estimate parasite growth, cell lines are built that are infected with DNA constructs carrying the luciferase system. In each of the cell lines a specific promoter is added to the luciferase system, either the \textit{ama}-1 promoter that is schizont-specific and therefore to be active only in schizont-stage parasites, or the eef1aa, which is a constitutive promoter that is expected to be working all times. The first system (\textit{ama}-1 promoter) is an \textit{In vitro} assay used to evaluate a drug effect on schizont growth, whereas a second system (\textit{eef} 1\textit{aa}) is an \textit{In vivo} test evaluated through analyzing drug effects on parasite growth during all stages [138].

14. \textit{In vivo} ANTIPLASMODIAL ASSAY

In the second step of the process, animal models, usually rodents (mice, young rats) are used to test the drug efficacy. In this step, species-specific parasites such as \(P. berghei\) are used according to a 4-day suppressive test. The experimental groups are treated with antiplasmodial agent at 0, 24, 48, and 72h post-infection of the animal models with \(P. berghei\). Tail blood is usually taken to determine parasitaemia and haematological parameters are monitored daily during the first 7 days and thereafter every 2 days interval for a period of 2 weeks [137]. Percentage parasitaemia, packed cell volume, hemoglobin, red blood cells count, white blood cells count and differential leucocyte count are monitored
during the period. Rodent malaria parasites have been proven to be analogous to the malaria parasites of man and other primates in most essential aspects of structure, physiology and life cycle [140].

15. ANTI-MALARIAL RESISTANCE

In Madagascar, there has been a shift with indoor residual spraying from DDT to pyrethroids, despite the two having similar efficacy. The two greatly decreased the vector-human contact with an associated decrease of the plasmodial index [141]. But the indoor biting behavior of Anopheles farauti documented 20 years ago in Guadalcanal still exist due to the failure of eradication programme [142].

The rapid spread of resistance to antimalarial drugs present a potentially devastating threat to effective safe treatment with effective and affordable options quickly running out. The discovery of new antimalarial drugs is not keeping pace. For decades chloroquine was the main drug of choice for treatment, however increasing resistance forced its replacement in parts of Asia and South America during the 1980s and in the African countries in the 1990s [13]. In Eastern Nigeria 40-60% of malarial cases have been reported not to respond to treatment with the drug [71]. There is evidence that chloroquine-resistant Plasmodium falciparum accumulates significantly less chloroquine than susceptible parasites by an accelerated drug efflux [143-145]. Various drugs, including calcium channel blockers (e.g. verapamil) and tricyclic compound (e.g. desipramine) have been shown to reverse or modify chloroquine resistance in vitro [146,147]. The decline in efficacy of Artesunate and Mefloquine concern the thai-Cambodian and Thai-Myanmar boarders. High prevalence of chloroquine and mefloquine-resistant P. falciparum isolates was observed during 2006–2009 in the area. Artesunate sensitivity declined and quinine sensitivity improved. Pfmdr1 and Pfmdr1 are the key genes that modulate multi-drug resistance in P. falciparum [125]. The persistence of malaria as a public health problem is partly as a result of resistance of malarial parasites to antimalarial drugs and to insecticides by Anopheles mosquitoes. Unfortunately, progress in the field of vaccine production has been slow although, a breakthrough by Patarroyo et al. has raised some hopes, however its usefulness to malaria endemic third world countries is yet to be determined [15].

16. CONTROL OF MALARIA

16.1 Development of Malaria Vaccines

Malaria vaccines are considered among the most important modalities for potential prevention of malaria diseases and reduction of malaria transmission. Upon all the intensive effort being made toward this area, there is currently no licensed malaria vaccine [148].

Attempts are now being made to improve the immunogenicity of antisporetozoite vaccines by taking into account, the requirement for T-cell as well as B-cell stimulation. Hitherto vaccines have been designed with blocking antibody in mind, but it has recently been shown that cytotoxic T cells can also be effective against the liver stage. However, the T cells of many patients living in endemic areas respond poorly or not at all to sporozoite antigens, presumably because parasites have selected antigenic variants that lead to their own survival [85]. Genetic studies have identified several loci correlated with severity of malaria [149]. Polymorphisms at the HLA loci which encode proteins that participate in antigen presentation, influence the course of malaria. In West Africa an HLA class I antigen (HLA
Bw 35) and HLA class II haplo type (DRB1*1302–DQBI*0501) are independently associated with protection against malaria. However, HLA correlations vary according to genetic constitution of polymorphism and geographic location [150].

Vaccine produced against *Plasmodium falciparium* injected intramuscularly into 40 volunteers produced antibody response only in 9 persons. The challenge of the 9 persons by mosquito bites indicated that only 2 were protected. Unfortunately, since a single surviving sporozoite can initiate clinical malaria, protection against this stage has to be “all or nothing” [150].

In another trial, a Colombian group was tested with a more complex antigen made by hybridization and then polymerising three separate peptides from the asexual blood parasites, which is the stage associated with the symptoms and lesions of malaria. Three of 9 volunteers, when challenged with parasites intravenously, had only transient low-level parasitaemias while the others required chemotherapy when their parasitaemia reached the previously agreed level of 0.5% [15]. The experiment highlights the ethical difficulties of testing this type of vaccine. A much larger trial of this strategy is now in progress with the precise role of T-cell stimulation under close scrutiny and there is no universal agreement about the sort of immunity that should be aimed for [151]. One worrying aspect is that many of the most promising parasite surface antigens are extremely polymorphic, so that a vaccine might have to contain antigens from numerous strains (as with the pneumococcal polysaccharide vaccine). It is interesting to note that, as with many parasites, some of the protective antigens are functional enzymes, whose aminoacid sequences may be expected to be less variable. An advantage of the blood stage is that protection does not have to be 100% effective to benefit the patient. Moreover there are a very large number of antigens to choose from, by no means all of which have been explored [152]. Due to the role that malaria parasite P_12 and P_38 proteins seem to play during invasion in *P. species*, added to the PV_12 and PV_38 antigenic characteristics and the low genetic diversity observed these proteins might be good candidates to be evaluated in the design of a multistage/multi-antigen vaccine [153].

Two other approaches, not yet tried in human populations, should also be mentioned. The first is the “transmission-blocking” vaccine, aimed at preventing the development of the sexual stage of the parasite in the mosquito. Vaccines consisting of gamete-derived antigens are surprisingly effective in animal models though there are many problems to overcome, one being the hint that suboptimal levels of immunity may actually enhance the production of infective sporozoite in the mosquito. This type of vaccine can only be evaluated at the population level, and both uptake and effectiveness would probably need to be high for any impact on the level of transmission [154].

The principal idea is to vaccinate against the disease rather than the parasite, rather in the same way as one does against tetanus and diphtheria, where it is the toxin and not the bacterium that causes the damage. No direct toxin has been isolated from the malaria parasite, but there is growing evidence for indirect toxicity via the overinduction of cytokines, particularly tumour necrosis factor (TNF), which has been implicated in the pathological changes in the brain, lung, liver, and other organs in severe malaria. If the molecules that induce TNF production can be identified, antibodies against them might form the basis of an “anti-disease” vaccine [152]. In summary, it seems certain that several more field trials will be necessary to evaluate the various strategies now under consideration, and it may be that in the end some kind of “cocktail” of the strongest antigens of each kind will give the best all-round results [151]. Unfortunately, Malaria Vaccine Technology Roadmap has been trying to
actualize its vision by developing effective vaccine against *P. falciparum* [155]. Can it make a landmark by developing and licensing a first generation malaria vaccine that will have a protective efficacy of more than 50% and lasts longer than one year by 2030? If not, the strategic goal of developing and licensing a malaria vaccine that has a protective efficacy of more than 80% against clinical disease and lasts longer than four years cannot be achieved! However, about 70 malaria vaccines have been reported, 7 (SR. 11.1, Pfs 25-EPA, CSVAE, ChAd63/MVA ME-TRAP+Matrix MTM polyepitope DNA EP1300 PiCellTOS FMP 012, and ChAd63 AMA/MVA AMAI+Al/CP67909) at the level of phase 1a, whereas 8 (Ad35.CS/RTS.S-A OI, Ad35.C5/Ad26.C5, ChAd63/MVA (CS; ME-TRAP), PiSPZ, PiGAPP52/-P32, ChAd63/MVA MSPI, ChAd63/MVA AMAI, FMP2.1–ASOIB (AMAI 3D7), NMRC. M3V.Ad. PIAE, and NMRC. M3V.D/Ad pICA) are at the phase 2a, but 6 (AD 35.C5, AMAI-CI-Alhydrogel+CPG 7909, SE36, BSAM-2Alhydrogel+CPG 7909, EBA175.R2, and CSP, AMAI (PEV 301, 302) at phase 1b level and so those at phase 2b level are GMZ2, MSP3 [181-276], and ChAd63/MVA MF-TRAP. Phase 3 vaccine being developed is RTS.S, A501 [153]. Development and phase 3 testing of the most advanced malaria vaccine, RTS, S/AS01, indicates that malaria vaccine R & D is moving into a new phase that will impact host-parasite relationship through vaccine-induced immune responses to multiple antigenic targets using different platforms [156].

**16.2 Campaign against Malaria**

Despite concerns that the feasibility of text-messaging interventions targeting caregivers may be compromised in rural high malaria risk areas in Kenya, very favourable conditions were found with respect to mobile network, access and ownership of phones, use of text-messaging and minimum literacy levels required for successful intervention delivery. More there was a high level of willingness of caregivers to receive text-message reminders. Impact evaluations of carefully tailored text-messaging interventions targeting caregivers of children with malaria are timely and justified [157].

In some parts of Africa, e.g., South-Sudan, the observed high level of malaria prevalence could be due to low levels of coverage and utilization of interventions coupled with low knowledge levels. Therefore, access and utilization of malaria control tools should be increased through scaling up coverage and improving behavior change communication [158]. Which types of behaviour change communication that can be accepted by all the endemic malaria countries?

In jiangsu province of China, there was a consistent increase in the number of malaria cases imported from other countries while the number of locally acquired cases sharply declined. This trend may be ascribed to the increasing investment from China to Africa and the rising number of Chinese labourers working in Africa. Therefore preventive efforts should be targeted on this high-risk group and the surveillance and response system should be strengthened to prevent local resurgence [159]. In Tanzania, there is a major shift in Anopheles gambiae S.I. sibling species composition combined with decline in overall vector density in the area. The decline has been most marked for Anopheles gambiae S.S., and least for Anopheles arabiensis. Due to differences in biology and vectorial capacity of Anopheles gambiae S.I. Complex, the change in sibling species composition will have important implications for the epidemiology and control of malaria and lymphatic filariasis in the study area [160].

Clusters of AMA-1 seroprevalence or parasite prevalence that are predictive of malaria infection after one year can be identified using geospatial models kernel smoothing using
1km window and spatial scan statistics both provided accurate prediction of future infection [161].

In addition to the availability of subsidized artemisinin-combination therapy, the intensity of communication campaigns may contribute to reported levels of the affordable medicines facility-malaria awarenesss and knowledge among private for-profit providers. Future subsidy programmes for antimalarials should similarly include communication activities [162]. Although, the malarial epidemiological and control status has changed markedly since 2006 when the Roadmap was originally launched [163].

The necessity of re-energizing basic research of malaria life-cycle and plasmodium developmental biology to provide the basis for promising and cost-effective vaccine approaches and to reach eradication goals is more urgent than previously believed. The focus of the field must be shifted to the basic research efforts including findings on the skin stage of infection [164]. A significant obstacle to malaria elimination in Asia is the large burden of *Plasmodium vivax* which is more difficult to eliminate than *plasmodium falciparum*. Persistent *P. vivax* liver stages can be eliminated only by radical treatment with a seven-day course of an 8-aminoquinoline, with the attendant risk of acute haemolytic anaemia with G6PD deficiency. In Azerbaijan, Tajikistan, North Afghanistan and DPR Korea 8,270,185 people received either a 14-day “standard” or a 17-day “interrupted” primaquine preventive treatment to control post-eradication malaria epidemics. Despite G6PD prevalence of up to 38.7%, the reported frequency of severe adverse reactions related to promaquine was very low [165].

16.3 The Use of Insecticide

Insecticide resistance is well established in malaria vectors throughout Africa and represents a threat to malaria control. In agriculture areas, the massive usage of pesticides from various families appears to select for metabolic and cuticle resistance mechanisms to a wide range of insecticides including pyrethroids and carbamates [166].

Several anopheline species occur in the northern Kruger National Park and their densities fluctuate between seasons. Species abundance and relative proportions within the Anopheles gambiae complex varied between collection method. There is a perennial presence of Anopheles arabiense in Malahlapanga site which declined in density during the dry winter months [167]. The use of pyrethroids to impregnate mosquito nest has had a good impact on the incidence of morbidity and mortality from malaria. These nets are therefore likely to be used on a large scale as an important strategy of malaria control in the future. In Malawi and Cameroon, the per household expenditure for impregnated mosquito nets compares favourably with the cost of malaria. The economic losses from malaria would be reduced by 37.83% over 3-year period in Malawi and in Cameroon savings 9.3% and 11.2% in two places resulted as a consequence of a diminished need for case treatment [168]. Both Kitibina and Folonzo chromosomal forms of Anopheles funestus are formidable malaria vector in Burkina Faso. However, the significantly greater tendency for the Kiribina form to rest outdoors despite its pronounced anthropophily suggests that uniform exposure of the overall Annopheles funestus population to indoor-based vector control tools cannot be expected. Kiribina is more likely to evade indoor intervention and escape unharmed outdoors, reducing the efficacy of malaria control [169]. But vaccinating Malawian children with RTS, S vaccines was very cost effective [170].
Insecticide-treated bed nets are preeminent malaria control means: though there is no consensus as to a best practice for large scale insecticide-treated bed net distribution. Studies revealed consistent inequalities between urban and rural populations; which were most effectively alleviated through a free-insecticide-treated bed net delivery and distribution framework [171].

16.4 The Use of Synthetic and Herbal Based Drugs

The major MSP-1\textsubscript{19} haplotypes of \textit{P. falciparum} population in all endemic populations in Thailand were identified, providing basic information for malaria vaccine development [172].

Idiopathic myocarditis has been reported during treatment for controlled human malaria infection [173]. Mass anti-malarial drug administration should be undertaken just before and during the rainy season when community members are less mobile [174]. The world’s first potential malaria vaccine proved only 30\% effective in African babies in crucial trial, calling into question whether it can be a useful weapon in the fight against the deadly disease [175].

OMARIA is prophylactic and therapeutic against stage of the parasite development and in patients having multiple infections. It has synergistic and buffering roles [176]. In the ancient Hindu scriptures, there had been mention of OMARIA which is widely available and is very cheap to produce [177]. Because women are more aware of children’s vulnerability to malaria, they are more inclined than men to want to buy permethrin impregnated bed nets [178].

\textit{Punica granatum} L. fruit is used in India for treatment of \textit{P. falciparum} and \textit{P. vivax} malaria [179]. Elagitanins have been responsible for inhibition of the proinflammatory mechanisms involved in the onset of cerebral malarial [180]. OMARIA-T cleared haemoproteza and wane of myalgia within 36 hours and conferred prophylaxis for months thereafter. But OMARIA-P completed the year prophylaxis with 99\% success devoid of side effects [181]. The \textit{In vitro} antiplasmodial activity of \textit{P. granatum} support its folkloric uses in the Asian continent as anti-malarial plant [181]. The sun dried dermis powder of \textit{Punica granatum} has been in continuous by Indian Red Cross, Koraput since June, 1998. The plant has prophylactic activity at the dose 1 gramme and therapeutic activity at the dose of 2 gramme [182]. The cytokine approach to disease pathogenesis can be said to have eventually reconciled Claude Bernard’s opinion that all disease conditions were disturbances of patients’ internal cellular environment and Louise Pasteur’s subsequent view that each disease was, instead, the direct result of specific microbial invaders and toxins [183] as seen in the case of malaria infection. Since malaria is changing its facet from time to time: with the change of human factors and the natural causes, the control efforts must be adapted accordingly. \textit{P. falciparum} malaria is the cause of all the mortality and most of morbidity in malaria. It can present with a typical features, cause dramatic complications and treatment may be rendered difficult by resistance to antimalarial drugs [184]. Also, the additional demonstration of microsatellite loci in \textit{P. vivax} as neutral markers capable of distinguishing the origin of the recurrent parasite (new infection or originating from the patient) lends support to their use in assessment of treatment outcomes [185]. The mechanism of massive intravascular haemolysis occurring during the treatment of malarial infection resulting in haemoglobinuria, commonly known as blackwater fever (BWF) remains unknown. BWF is most often seen in those with severe malaria treated with amino alcohol drugs including quinine, mefloquine and halofantrine. The potential for drugs containing artemisinin, chloroquine or \textit{Piperawuine} to cause oxidant haemolysis is believed to be much lower, particularly during treatment of uncomplicated malaria. BWF was reported to have developed on day two of treatment for uncomplicated \textit{Plasmodium falciparum} infection with dihydroartemisinin-piperaquine with documented evidence of concomitant seropositivity for
chikungunya infection [186]. Another challenge against control of malaria is the emergence of *Plasmodium vivax* which is increasingly recognized as being capable of causing severe disease. But evidence supporting the occurrence of severe infection is rare. However, reported is a case of severe *P. ovale* infection in a patient presenting with jaundice, respiratory distress, severe thrombocytopenia, petechiae, and hypotension [187]. In addition, there are sever neglected problems with antimalarial quality, but there are important caveats to accurately estimate the prevalence and distribution of poor quality antimalarials. But lack of reports in many malaria-endemic areas, inadequately sampling techniques and inadequate chemical analytical methods and instrumental procedures emphasizes the need to interprete medicine quality results with caution. The available evidence demonstrates the need for more investment to improve both sampling and analytical methodology and to achieve concensus in defining different types of poor quality medicine [188]. But pharmacological modelling of real-life scenarious can provide valuable supportive data and highlight modifiable determinants of therapeutic effectiveness that can help optimize the deployment of anti-malarials in control programmes [189].

17. CONCLUSION

Because of the long recognition of malaria through anthropology, a lot was known about control and treatment of malaria. But development of vaccines is not keeping pace due to antigenic shift of all the stages of *Plasmodium*, in vitro and vivo antiplasmodial assays need to be improved upon with a view to proffering solution to episode of malaria resistance. In the local areas where malaria is sporadic and endemic, there is need to combine chemotherapy with ethnomedicine in addition to the new available vaccines, since many malaria patients from developing countries cannot afford costlier effective and less toxic antimalarials.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ukoli FMA. Introduction of Parasitology in Tropical Africa, Chichester: John Wiley and Sons Ltd. 1984;404.
2. Ukoli FMA. The biology and natural history of malaria. Proceedings of the fifth annual convention and scientific assembly. Arch Ibad Med. 2003;1(2):35-36.
3. Katzung B. Basic and Clinical Pharmacology. International edition. London: McGraw Hill London. 2004;864–873.
4. Bruce-Chwat LJ. Essential Malariology. London: William Heinemann Medical Books Ltd. 1980;354.
5. Brabin BJ. Malaria in pregnancy. Its importance and control. Postgrad Doc. Afr. 1989;2:57-59.

6. Abd-Rabou I. Medicinal herbal research in West Africa. In proceedings of the 24th Annual Regional Conference of the West Africa Society for Pharmacology (WASP): 22 - 25 October, 1997, Usmanu Danfodiyo University, Sokoto. 1997;2-3.

7. WHO. Promotion and development of training and research in traditional medicine. Technl Report Geneva. 1997;622.

8. Rabo ET, Sanusi SS. An Inventory of Medicinal Plants of the Nigerian Savannah, Lagos: Leviathan Books. 2001;21-24.

9. Wambebe C. Development and production of standardized phytomedicines. West Afr J Pharm. 1998;12(2):13-24.

10. WHO. The promotion and development of traditional medicine. Techn Research Ser Geneva. 1992;143.

11. Ghirotti M. The possible role of ethnoveterinary medicine in the delivery of more effective animal health services in tropical and sub-tropical areas. Giornate Halianio Dimedicine Tropicale. 1996;1:34-39.

12. WHO. World malaria report. WHO Bull. 2008;99.

13. WHO. Rolling back malaria. The World Health Report. 1999;49.

14. The Health Exchange. Roll back malaria facing the challenge in complex emergencies. 2001;62.

15. Patarroyo ME, et al. A synthetic vaccine protects human against challenging with asexual blood stage of plasmodium falciparum malaria. Nature. 1988;332:158–161.

16. Iwu MM. Handbook of African Medicinal Plants. London; CRC Press, Boca Raton. 1993;12-14.

17. Saganuwan SA, Yatswako S. Malaria parasites of clinical and laboratory importance—an update. J Med Pharmaceut Sci. 2006;2(3):96–105.

18. Hackskaylo MM. Culinary garden. In The National Herb Garden. Edited by Ober R. Springfield; The Herb Society of America. 1996;79–93.

19. Davidson A. The Oxford Companion to Food. Oxford:Oxford University Press; 1999.

20. Remillard RL, Wynn SG. Herbal medicines In Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat. 6th edition. Edited by Etinger, SJ, Feldman FC. Philadelphia: Elsevier. 2005;524-526.

21. Hoareau L, Edgar JD. Medicinal plants: A re-emerging health aid. Electr J Biotechnol 1999;2(2):56–70.

22. Bateman DN, Dyson EH. Quinine toxicity: Adverse drug reaction. Acute poison Rev. 1986;5:215-233.

23. Johann-Liang R, Albracht R. Safety evaluations of drugs containing artemisinin derivatives for treatment of malaria. Clin Infect Dis. 2003;36:1626-1627.

24. Tella A. The effects of Azadirachtta indica in acute Plasmodium berghei malaria. Nig Med J. 1965;7(3):258–263.

25. Mann A, Gbate M, Nda Umar A. Medicinal and Economic Plants of Nupeland, Bida: Jube Evans Books and Publications. 2003;275.

26. Luize PS, et al. Effects of medicinal plant extracts on growth of Leishmania (L.) amazonensis and Trypanosoma cruzi. Braz J Pharmaceut Sci. 2005;14(1):85–94.

27. De Mesquita ML, et al. In vitro antiplasmodial activity of Brazilian Cerrado plants used as traditional remedies. J. Ethnopharmacol. 2007;110(1):165–170.

28. Devkota A, Jha PK. Biology and medicinal characteristics of Centella asiatica. In: Jha, P.K., Karmacharya, S.B., Chettri, M.K., Thapa, C.B. and Shrestha, B.B. (eds) Medicinal plants in Nepal. An Anthology of Contemporary Research Ecological Society (ECOS), Kathmandu. Edited by Karmacharya SB, Chettri MK; Thapa CB, Shrestha BB; 2008.
29. Laouer H, et al. Chemical composition and antimicrobial activity of essential oil of Bupleurum montanum and bupleurum plantagineum. Nat Prod Commun. 2009;4(11):1605-1610.
30. Esmaeili S, et al. Screening of antiplasmodial properties among some traditionally used human plants. J Ethnopharmacol. 2009;121(3):400-404.
31. Dhar ML et al. Screening of Indian plants for biological activity. Ind J Exper Biol. 1968;6(4):232–247.
32. Okokon JE, Ubolum PM, Udokpoh AE. Antiplasmodial activity of Setaria megaphylla. Phytother Res. 2007;21(4):366–368.
33. Waaka PJ, et al. The in vitro and in vivo antimalarial activity of Cardiospermum halicacabum L. and Momordica foetida Schundi Et Thonn. J Ethnopharmacol. 2005;99(1):137–143.
34. Amorim CZ, Marques AD, Cordeiro RS. Screening of the antimalarial activity of plants of the Curcurbitaceae family. Mem Inst Oswaldo Cruz. 1991;86(2):177–180.
35. Mustofa, Sholikhan EN, Wahyono S. In vitro and in vivo antiplasmodial activity and cytotoxicity of extract of phyllanthus niruri L. herbs traditionally used to treat malaria in Indonesia. Southeast AS J Trop Med Public Health. 2007;38(4):609–615.
36. Malebo HM, et al. Antiprotozoal activity of aporphine and protoberberine alkaloids from Annickia Kummeriae (Eng. & Diets) Setten & Maas. Complement Alternat Med. 2013,13:48. Doi: 10.1186/1472-6882-13-48.
37. Duker-Eshun G, et al. Antiplasmodial constituents of Cajanus cajan. Phytother Res. 2004;8(2):128–130. Doi: 10.1002/ptr.v18:2/issuetoc.
38. Coker HAB, Adesegun SA. The malaria scourges the place of complementary traditional medicine. Nig Med Pract. 2006;49(5):126-132.
39. Gupta SS. Prospects and perspective of natural products in medicine. Ind J Pharmacol. 1994;26:1-12.
40. Saganuwuan SA. Some tropical plants with antihypertensive, antiasthmatic and antidiabetic value. J Herb Spice Med Plant. 2009;15(1):24-44.
41. Geidam YA. Antibacterial and toxicological studies of aqueous leaf extract of Psidium guajava (guava) in Chickens. Ph.D. Thesis, University of Maiduguri, Maiduguri, Nigeria; 2008.
42. Saganuwan SA, Gulumbe ML. Evaluation of In-vitro antimicrobial activities and phytochemical constituents of Cassia occidentalis. ARI. 2006;3(3):566–569.
43. Saganuwan SA. Toxicological and antimalarial effects of aqueous leaf extract of Abrus precatorius (Jecquirity bean) in Swiss albino mice. Ph.D. Pharmacology Thesis, Sokoto: Usmanu Danfodiyo University. 2012:242.
44. Tracy JW, Webster. Drugs used in the chemotherapy of protozoal infections. In Goodman and Gilman’s The pharmacological Basis of Therapeutics. Edited by Hardman JG, Limbird LE, Mohinstt PB, Ruddon RW, Gilman AG. Ninth edition. McGraw Hill Companies. 1996;1905.
45. Smyth JD. Animal Parasitology. Cambridge low price edition. Cambridge: University of Cambridge Press. 1996;126-136.
46. Bradley DJ. The last and the next hundred years malariology. Parasitologia. 1999;41:11-18.
47. Laveran C. Pludism. In Trans. Edited by Martins JW. London: The New Sydenham Society. 1983;197.
48. MacCallum WG. On the flagellated form of the malaria parasite. Lancet. 1897;2:1240-1241.
49. MacCallum WG. On some peculiar pigmented cells found in two mosquitoes fed on malaria blood. Br Med J. 1897;2:1786-1788.
50. Ross R. On the haematozoan infections of birds. J Exp Med. 1897;3:117-136.
51. Ross R. Researches on malaria. JR Army Med Corps. 1905;4:474.
52. Kettle DS. Medical and Veterinary Entomology, Wallingford: CAB International. 1993;513-541.
53. WHO. Malaria actions: Programme, severe and complicated malaria. Tran Royal Soc Med Hyg. 1986;80:1-50.
54. Laloo DG, et al. UK malaria treatment guidelines. J Infect. 2006;54:111–121.
55. WHO. Roll back malaria: A global partnership. WHO Geneva; 1995.
56. Cheesebrough M. District Laboratory Practice in Tropical countries Part 1. Low price edition. Cambridge University Press. 2005;434.
57. Disease Watch. Malaria. TDR Nature Reviews Microbiology in District laboratory practice in Tropical Countries. Edited by Cheesebrough M. Cambridge: Cambridge University press. 2004;454.
58. Dellacolle C. Malaria epidemic response: What the clinician needs to know. Mera. 2004;3.
59. Ukoli FMA. Prevention and control of parasitic diseases in tropical Africa: The main issues, Ibadan: University Press Plc. 1992;192.
60. Supargiyano S, et al. Seasonal changes in the antibody responses against plasmodium falciparum merozoite surface antigens in areas of differing malaria endemicity in Indonesia. Malaria J. 2013;12:444.
61. Gilles HM. Diagnostic methods in malaria. In Bruce-Chawtt Essential Malariaology. Third edition. Edited by Gilles HM, Warrell DA, Great Britain: Division of Holder Stoughton. 1993;78-95.
62. Akubue PI. Malaria. In Textbook of Pharmacology. Edited by Akubue PI. Onitsha: Africana first publishers limited. 2006;536.
63. James DM, Gilles HM. Human Antiparasitic Drugs: Pharmacology and Usage, John Wiley & Sons. 1985;120.
64. Kebede S, et al. Re-evaluation of microscopy confirmed Plasmodium falciparum and plasmodium vivax malaria by nested PCR detection in Southern Ethiopia. Malaria J. 2014;13:48. Doi: 10.1186/1475-2875-13-48.
65. Gething PW, et al. Declining malaria in Africa: Improving the measurement of progress: Malaria J. 2014;13:39. Doi: 10.1186/1475-2875-13-39.
66. Bennett PN, Brown MJ. Protozoal infection. Clinical Pharmacology Edited by Bennet PN, Brown MJ. New Delhi: Churchill Livingstone. 2005;789.
67. Nester EW, et al. Microbiology: A Human Perspective. Fourth edition, New York; McGraw Hill. 2004;731-738.
68. Shapiro TA, Goldberg DE. Chemotherapy of protozoal infections (Malaria). In: Goodman and Gilman’s, the pharmacological Basis of Therapeutics. Edited by Brunton LL, Lazo JS, Parker KL, New York: McGraw Hill Medical Publishing. 2006;1021–1045.
69. Rang HP, et al. Rang and Dale’s Pharmacology. 6th edition. Philadelphia: Churchill Livingstone. 2008;829.
70. Sibley L, Sipe TA, Koblinsky M. Does traditional birth attendant training improve referral of women with obstetric complications: a review of the evidence. Soc Sci Med. 2004;59(8):1757-1768.
71. Okolie VE, et al. Prevalence and risk factor for placental malaria in Nnewi, South East Nigeria. Int. J Trop Health Dis. 2014;4(3):374–383.71.
72. Van Buskirk KM, Sevova E, Adams JH. Conserved residues in the Plasmodium vivax. Duffy-binding protein ligand domain are critical for erythrocyte receptor recognition: Proc Natl Acad Sci USA. 2004;101:15754-9. PMC free article: PMCS24844, PubMed: 15498870.
73. Livingstong FB. The Duffy Blood groups, vivax malaria and malaria selection in human population: A review. Human Biol. 1984;56:413–425. PUBMed: 6386656.

74. Barbara JAJ. Microbiology in Blood Transfusion, London: Wright. PSG. 1983;97-103.

75. Craig A, Scherf A. Molecules on the surface of the Plasmodium falciparum infected erythrocytes and their role in malaria pathogenesis and immune evasion. Molecul Biochem Parasitol. 2001;115:129-143.

76. Ta TH, et al. First case of naturally acquired human infection with Plasmodium cynomolgi. Malaria J. 2014;13:68. Doi:10.1186/1475-2875-13-68.

77. Stanier RY, et al. The Microbial World. Fifth edition. New Jersey: prentice-hall, Englewood Cliffs. 1986;647-656.

78. Conrad ME. Diseases transmissible by blood transfusion: Viral hepatitis and other infectious diseases. Semin Haematol. 1981;18:122-146.

79. Lopez C, et al. Mechanisms of genetically based resistance to malaria. Gene. 2010;467(1-2):1–12. Doi:10.1016/j.gene.2010.07.008.

80. Anstee DJ. The relationship between blood groups and disease. Blood. 2010;115(23):4635-4643. PMID 20308598 (//www/nibi.nlm.gov/pubmed20308598).

81. May, et al. Hemoglobin variants and disease manifestations in severe falciparum malaria. JAMA. 2007;297(20):2220–2226. PMID17519411.

82. Allen SJ, et al. Prevention of cerebral malaria in children in Papua New Guinea by South East Asian ovalocytosis band 3”. Am J Trop Med Hyg 1999;60(6):1056–1060. PMID10403343.

83. Cortes A, et al. Adhesion of Plasmodium falciparum-infected red blood cells to CD36 underflow is enhanced by the cerebral malaria-protective trait South-East Asian ovalocytosis”. Mol Biochem Parasitol. 2005;142(2):252–257. PMID 15978955.

84. Terrenato L, et al. Decreased malaria morbidity in Tharu people compared to sympatric people populations in Nepal. Ann Trop Med Parasitol 1988;82(1):1-11. PMID3041928.

85. Schofield L, Grau GE. Immunological process in malaria pathogenesis. Nature reviews. Immunology. 2005, 5(9):722–735. PMID 16138104.

86. Viser BJ, et al. Serum lipids and lipoproteins in malaria-a systematic review and meta-analysis. Malaria J. 2013;12:442.

87. Lau TY, Sylri M, William T. Mutational analysis of Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase in the interior division of Sabah, Malaysia. Malaria J. 2013;12:445.

88. Martins AC, et al. Vivax malaria in an Amazonian child with dilated cardiomyopathy. Malaria J. 2014;13:61. Doi: 10.1186/1475-2875-13-61.

89. Hardman JG, et al. Goodman and Gilmen's. The Pharmacological Basis of Therapeutics 9th edition. London: McGraw Hill. 1996;1905.

90. Zhao X, et al. The temporal lagged association between meteorological factors and malaria in 30 countries in South-West China: A multilevel distributed lag non-linear analysis. Malaria J. 2014;13:57. Doi: 10.1186/1475-2875-13-57.

91. Philips RS. Current status of malaria and potential. Clin Microbiol Rev. 2001;14(1):208-226.

92. Witworth J, et al. Effect of HIV and increasing immunosuppression on malaria parasitaemia and clinical episodes in rural Uganda adults: A cohort study. Lancet 2000;356:1051-1056.

93. Francesconi, et al. HIV, malaria parasites and acute febrile episodes in Ugandan adults: A case control study. AIDS. 2001;15:2445-2450.

94. Bastos F, et al. Co-infection with malaria and HIV in injecting drug user in Brazil: A new challenge to public health. Addict. 2004;94(8):1165-1174.
95. NIH National Institute of Health Clinical Centre. Medical Implications of Co-Infection with Malaria and Filariasis Parasites—Full Text View—Clinical Trials. Gov processed this record on February 13, 2014. Available: http://clinicaltrials.gov/NCT00471666.

96. Sinden RE, et al. The biology of sexual development of plasmodium: The design and implementation of transmission blocking strategies. Malaria J. 2012;11:70. Available: http://www.malariajournal.com/content/11/1/70.

97. Prato M, Giribaldi G. Matrix metalloproteinase-9 and haemozoin: Wedding rigs for human host plasmodium parasite in complicated malaria. J Trop Med. 2011;1–11. Doi:10.1155/2011/628435.

98. WHO Lives at Risk: Malaria in pregnancy. 2014;1–3. Available at: www.whoint/feature/2003/04b/en.

99. Jeffery GM. Infectivity to mosquitoes of Plasmodium vivax and Plasmodium falciparum under various conditions. Amer J Trop Med Hyg. 1960;9:315-320.

100. Bray RS. Observations on the cytology and morphology of the mammalian malaria parasites. A process of apparent plasmotomy in the preerythrocytic phase of Laveranian falciparum. Riv Parasit. 1960;21:267-276.

101. Horsefall WR. Medical Entomology: Arthropods and Human Diseases; New York: The Ronald press Company. 1962;338-347.

102. Bhattacharya D. Relevance of economic field microscope in remote rural regions for concurrent observation of malaria and inflammation. Adv Infect Dis. 2012;1(2):13–18. Doi:10.1016/52222– 1808(11) 60055-8. Available: http://www.apitem.com/zz/2011jun/13.pdf.

103. Roobsoong W, et al. A rapid sensitive, flow cytometry-based method for the detection of Plasmodium vivax-infected blood cells. Malaria J. 2014;13:55. Doi: 10.1186/1475-2875-13-55.

104. Talundzic E, et al. Field evaluation of the photo-induced electron transfer fluorogenic primers (PET) real-time PCR for the detection of plasmodium falciparum in Tanzania. Malaria J. 2014;13:31. Available: http://www.malariajournal.com/content/13/1/31.

105. Hawkes M, et al. Use of a three-band HRP2/PLDH combination rapid diagnostic test increases diagnostic specificity for falciparum malaria in Uganda children. Malaria J. 2014;13:43. Available: http://www.malariajournal.com/content/13/1/43.

106. Tiono AB, et al. Lesson slearned from the use of HRP-2 rapid diagnostic test in community-wide screening and treatment of asymptomatic carriers of Plasmodium falciparum in Burkina Faso. Malaria J. 2014;13:30. Doi: 10.1186/1475–2875–13- 30.

107. Zuzarte-Luis V, Sales-Dias J, Mota MM. Simple, sensitive and quantitative bioluminescence assay for determination of malaria prepatent period. Malria J. 2014,13:15. Available: http://www.malariajournal.com/content/13/1/15.

108. Foster D, et al. Evaluation of three rapid diagnostic tests for the detection of human infections with plasmodium knowlesi. Malaria J. 2014;13:60. Doi:10.1186/1475-2875-13-60.

109. White NJ. In Manson’s Tropical Diseases Edited by Cook G. London: WB Saunders. 1996;1087-1164.

110. White N. Antimalarial drug resistance and combination chemotherapy. Philos Trans R Soc. 1997;354:739-749.

111. Neave PE, Jone COH, Behrens RH. Challenges facing providers of imported malaria—related healthcare services for Africans visiting friends and relatives (VFRs). Malaria J 2014;13:7. Doi: 10.1186/1475 – 2875-13-17.

112. WHO. Quality Control Method for Medicinal Plants Materials, Switzerland. 1998;115.

113. Adeoti OM, et al. Presumed malaria and human immunodeficiency disease: a challenge to health planners. J Med Pharmaceut. Sci. 2007;3(3):75-80.
114. Klayman DL. Qinghaosu (artemisinin): An antimalarial drug from China. Sci. 1985;228:1049-1055.
115. Namdeo AG, Mahadik K, Kadam SS. Antimalarial drug–Artemisia annua. Pharmacogn Magaz. 2006;2(6):106-111.
116. Jimmy ED, et al. Haematologic responses in comparative doses of chloroquine, fansidar, lapdap, amalar and cotexcin. J Med Pharmaceut Sci. 2006;2(4):1-4.
117. Rosenthal PJ. Basic and Clinical Pharmacology. Ninth edition, New Delhi: MC Graw Hill. 2004;1202.
118. Terkuile FO, et al. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. Am J Trop Med Hyg. 2004;71:41-54.
119. Exavery A, et al. Factors affecting uptake of optimal doses of sulphadoxine-pyrimethamine for intermittent preventive treatment of malaria in pregnancy in six districts of Tanzania. Malaria J. 2014;13:22. Doi: 10.1186/1475-2875-13-22.
120. Greenstein B, Gould D. Trounce’s Clinical Pharmacology for Nurses, 18th edition. Edinburgh: Churchill Livingstone. 2009;483.
121. Tatem AJ, et al. Integrating rapid risk mapping and mobile phone cell record data for strategic malaria elimination planning. Malaria J. 2014;13:52. Doi:10.1186/1475-2875-13-52.
122. Abrams AC. Clinical Drug Therapy, 7th edition, Philadelphia: Lippincott Williams and Wilkins, A. Wolters Klower Company. 2004;1032.
123. Tripathi KD. Essentials of Medical Pharmacology. 5th edition: New Delhi: Jaypee Brothers Medical Publishers (p) Ltd. 2003;735-748.
124. Carson PE, Flanagan CL, Ickes CE, Alving AS. Enzymatic deficiency in primaquine sensitive erythrocytes. Sci 1956, 124: 484 -485.
125. Phompradit P, et al. Four years’ monitoring of in vitro sensitivity and candidate molecular markers of resistance of plasmodium falciparum to artesunate-mefloquine combination in the Thai-Myanmar boarder. Malaria J. 2014;13:23. Doi: 10.1186/1475-2875-13-23.
126. Djalle D, et al. Efficacy and safety of artemether+lumefantrine, artesunate+ sulphamethoxypyrazine-pyrimethamine and artesunate+amodiaquine and sulphadoxine-pyrimethamine in the treatment of uncomplicated falciparum malaria in Bangui, Central African Republic: A randomized trial. Malaria J. 2014;13:9. Doi:10.1186/1475-2875-13-9.
127. Soh PN, et al. In vitro and In vivo properties of ellagic acid in malaria treatment. Antimicrob Agent Chemother. 2009;53(3):100–106. Doi:10.1128/AAC.01175-08.
128. Drug One, Why Fight Malaria? Chandigarh, India, May. 2007;2(9):30-36.
129. GFATM. The Global Fund to Fight Aids, Tuberculosis and Malaria, 2010. Available at: www.fightmalaria.org/uc-Dublin-2012.
130. UNICEF. White House Summit on Malaria New York, 2006. Available at: http://www.unicef.org/health/index_37773.html
131. Dow SG, et al. A retrospective analysis of the protective efficacy of tafenoquine and mefloquine as prophylactic anti-malarials in non-immune individuals during development malaria-endemic area. Malaria J. 2014;13:49. Doi: 10.1186/1475-2875-13-49.
132. Singh J, et al. Clinical manifestations, treatment and outcome of hospitalized patients with plasmodium vivax malaria in two Indian states: A restrospective study. Malaria Res Treat. 2013;1:1–5. Available: http://dx.doi.org/101155/2013/341862.
133. Bjorkman A, Phillips-Howard PA. Adverse reactions to sulfa drugs: Implications for malaria chemotherapy. Bull WHO. 1991;69:297-304.
134. Saganuwan SA. Palaver of *In vitro*–*In vivo* antiplasmodial activities correlation. BIT’s 3rd Annual World Congress of Microbes – 2013, Juuly 30 – August 7. 2013;345.

135. Coulibaly SO, et al. Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42 – day *In vivo* follow-up study. Malaria J. 2014;13:41. Available: http://www.malariajournal.com/content/13/1/41.

136. Franke-Fayard B, et al. Simple and sensitive antimalarial drug screening *In vitro* and *In vivo* using transgenic luciferase expressing plasmodium berghei parasites. Int J Parasitol. 2008;20:1–5.

137. Bahamontes–Rosa N, et al. *In vivo* anti-malarial effect of β-amino alcohol 1t on Plasmodium berghei. Parasitol Res. 2009;10:1-9.

138. Timothy GG, Divo AA, Jensen JB. An *In vitro* assay system for the identification of potential antimalarial drugs. J Parasitol. 1983;69(3):577-583.

139. Lekana-Douki JB, et al. Indian antimalarial OMARIA is effective against African drug resistant P. falciparum field isolates and laboratory strains; without toxicity. Int J Clin Med. 2012;3:1-8. Doi:10.4236/jcm.2012.31001.

140. Carter R, Diggs CL. Plasmodium of rodents. In Parasitic Protozoa Volume III. Edited by Kreier JP. New York: Academic press. 1977;359-465.

141. Ratovonjato J, et al. Entomological and parasitological methrin in the Western forthill area of Madagascar. Malaria J. 2014;13:21. Doi: 10.1186/1475-2875-13-21.

142. Bugo H, et al. The bionomics of the malaria vector anopheles farauti in Northern Guadal canal, Solomon Islands: Issues for successful vector control. Malaria J. 2014;13:56. Doi: 10.1186/1475-2875-13-56.

143. Krogstad DJ. Efflux of chloroquine from *Plasmodium falciparum*: Mechanism of chloroquine resistance. Sci. 1987;238:1283-1285.

144. Crownman AF, Karecz. Drug resistance and the P-glycoprotein in homologues of *Plasmodium falciparum*: Semin cellbiol. 1993;4:29-35.

145. Olliaro PL, Trigg PL. Status of antimalaria drugs under development. Bull WHO. 1995;73(5):565-571.

146. Bitonti AJ. Reversal of chloroquine resistance in malaria parasite *Plasmodium falciparum* by desipramine. Sci. 1988;242:1301-1303.

147. Martins SK, Oduola AMJ, Mkilhous WK. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamill. Sci. 1987;242:1301-1303.

148. WHO. Malaria Vaccine Rainbow Tables. 2012. Available at: www.who.wit/vaccine_research/en/Rainbowindex.html.

149. Frodsham AJ, Hill AV. Genetics of infectious diseases. Hum Mol Genet. 2004;13(2):187–194. PMID 15358724.

150. Hill AVS, et al. Common West African HLA antigens are associated with protection from severe malaria. Nature. 1991;353(6336):595–600.

151. Playfair JHL, Blackwell JM, Miller HRP. Parasitic diseases. In Modern Vaccines, Current Practices and New Approaches. Edited by Moxon ER, Great Britain: Edward Armed. 1990;150.

152. Playfair JHL, et al. The malaria vaccine: anti-parasite or anti-disease? Immunol Today. 1990;11:25-27.

153. Forero-Rodriguez J, Garzon-Ospina D, Patarroyo MA. Low genetic diversity and functional constraint in loci encoding plasmodium vivax p12 and p38 proteins in the Colombian population. Malaria J. 2014;13:58. Doi:10.1186/1475-2875-13-58.

154. Mendis KN, et al. Malaria transmission-blocking immunity induced by natural infections of *Plasmodium vivax* in humans. Infect Immun. 1987;55:369-77.
155. Malaria Vaccine Technology Roadmap Final Report 2006. Available at: http://www.malariavaccine.org/files/Malaria_Vaccine_TRM_Final_ooo.pdf.

156. Schwartz L, et al. A review of malaria vaccine clinical projects based on the WHO rainbow table. Malaria J. 2012;22:22. Doi: 10.1186/1475-2875-11-11.

157. Otieno G, et al. The feasibility, patterns of use and acceptability of using mobile phone text-messaging to improve treatment adherence and post-treatment review children with uncomplicated malaria in Western Kenya. Malaria J. 2014;13:44. Doi:10.1186/1475-2875-13-44.

158. Eyobo MB, et al. Malaria indicator survey 2009, South Sudan: baseline result at household level. Malaria J. 2014;13:43. Available: http://www.malariajournal.com/content/13/1/45.

159. Liu Y, et al. Malaria in overseas labourers returning to China: An analysis of imported malaria in Jiangsu province, 2001–2011. Malaria J. 2014;13:9. Available: http://www.malariajournal.com/content/13/1/29.

160. Derua YA, et al. Change in composition of the anopheles gambiae complex and its possible implications for the transmission of malaria and lymphatic filariasis in north-eastern Tanzania. Malaria J. 2012;11:188. Available: http://www.malariajournal.com/content/13/1/188.

161. Mosha JF, et al. Hot spot or not: a comparison of spatial statistical methods to predict prospective malaria infections. Malaria J. 2014;13:43. Doi: 10.1186/1475–2875-13-53

162. Willey BA, et al. Communicating the AMFM message: Exploring the effect of communication and training interventions on private for-profit provider awareness and knowledge related to a multi-country anti-malarial subsidy intervention. Malaria J. 2014;13:46. Doi:1186/1475–2875-13-46.

163. Malaria Vaccine Technology Roadmap, November. 2013;1–9.

164. Lorenz V, Karams P. Malaria vaccines: Looking back and lessons learnt. Asian Pac J Trop Biomed. 2011;1(1):74–78. Doi:10.1016/32221–1691 (11) 60072–5.

165. Kondrashin A, et al. Mass primaquine treatment to eliminate vivax malaria: Lessons from the past. Malaria J. 2014;13:51. Doi:10.1186/1475 – 2875-13-51.

166. Nkya TE, et al. Insecticide resistance mechanisms associated with different environments in the malaria vector Anopheles gambiae: A case study in Tanzania. Malaria J. 2014;13:28. Available: http://www.malariajournal.com/content/13/1/28.

167. Munhenga G, et al. Field study site selection, species abundance and monthly distribution of anopheline mosquitoes in the northern Kruger National Park, South Africa. Malaria J. 2014;13:27. Doi:10.1186/1475 – 2875-13-27.

168. Brinkman U, Brinkmann A. Economic aspects of the use of impregnated mosquito nets for malaria control. Bul WHO. 1995;73(5): 651–658.

169. Guelbeogo WM. Behavioural divergence of sympatric Anopheles funestus populations in Burkina Faso. Malaria. 2014;13:65. Doi:1031186/1475-2875-13-65.

170. Seo MK, Baker P, Ngo KN-L. Cost-effectiveness analysis of vaccinating children in Malaria with RTS, S vaccines in comparism with long-lasting insecticide-treated nets. Malaria J. 2014;13:66. Doi:10.1186/1475-2875-13-66.

171. Sexton AR. Best practices for an insecticide-treated bed net distribution programme insub-saharan eastern Africa. Malaria J. 2011;10:157. Doi:10.1186/1475-2875-10-157.

172. Simpalipan P, et al. Diversity and population structure of Plasmodium falciparum in Thailand based on the spatial and temporal haplotype patterns of the C-terminal 19-KDa domain of merozoite surface protein-1. Malaria J. 2014;13:54. Doi:10.1186/1475-2875-13-54.

173. Van Meer MPA, et al. Indiopathic myocarditis during treatment for controlled human malaria infection: a case report. Malaria J 2014;13:38. Available: http://www.malariajournal.com/content/13/1/38.
174. Dial NJ, et al. A qualitative study to assess community barriers to malaria mass drug administration trials in the Gambia. Malaria J 2014;13:47. Available: http://www.malariajournal.com/content/13/1/47.

175. Reiland K, Hirschler B. Setback for first malaria vaccine in African trial. Fri, Nova; 2012. Available: http://www.reuters.com/assets/printpaid:USBRE8A801120121109.

176. Bhattacharya D, et al. Transmission blocking of year round resistant malaria in Koraput (India) by OMARIA – a new antimalarial phytotherapy. 2013;3(1):54–77.

177. BBC News. Indian claims herbal malaria cure. South Asia, Tuesday, 24 October; 2000.

178. Rashed S, et al. Determinants of the epermethrin impregnated bednets (Pib) in the republic of Benin: The role of women in the acquisition and utilization of pibs. Soc Sci Med. 1999;49(8):993–1005.

179. Dell’ Agli M, et al. Antiplasmodial activity of Punica granatum L. fruit rind. J Ethnopharmacol. 2009;125(2):279–285.

180. Dell’ Agli M, et al. Ellagitannins of the fruit of Pomegranate (Punica granatum) antagonize the host inflammatory response mechanisms involved in the onset of malaria. Malaria J. 2010;9(1):208.

181. Bhattacharya D. Punica granatum’s dermis indicates antimalarial therapeutics and prophylaxis. 4th Pan African Malaria Conf Multilat Initiat on Malaria Yaounde, Cameroon 13 – 18 Nov; 2005.

182. Bhattacharya D. Tannins, ions, cations and malariais: observations and theory Am J Trop Med Heyg. 2007;77(5):27.

183. Clark IA, et al. Pathogenesis of Malaria and clinically similar conditions. Clin Microbial Rev. 2014;17(3):509–539. Doi:10.1128/CMR.17.3.

184. Panda M, Mohapatra A. Malaria control–an over view in India. J Hum Ecol. 2004;15(2):101–104.

185. McCollum AM, et al. Genetic variation and recurrent paraistaemia in Peruvian Plasmodium vivax populations. Malaria J. 2014;13:67. Doi:10.1186/1475-2875-13-67.

186. Lon C, et al. Black water fever in an uncomplicated Plasmodium falciparum patient treated with dihydroartemisin-piperaquine. Malaria J. 2014;13:96. Doi:10.1186/1475-2875-13-96.

187. Strudom KA, Ismail P, Frean J. Plasmodium ovale: A case of not-so-benign tertian malaria. Malaria J. 2014;13:85. Doi:10.1186/1475-2875-13-85.

188. Tabermero P, et al. Mind the gaps—the epidemiology of poor-quality anti-malarials in the malarious word-analysis of World Wide Antimalarial Resistance Network database. Malaria J. 2014;13:139. Doi:10.1186/1475-2875-13-139.

189. Hodel EM, et al. Optimizing the programmatic deployment of the anti-malarials artemether-lumefantrine and dihydroartemisinin-piperaquine using pharmacological modelling. Malaria J. 2014;13:138. Doi: 10.1186/1475-2875-13-138.

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