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Anti-malarial treatment: Herbal medicine a ray of hope

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Abstract

For years, conventional medicines have been broadly used for treatment of malaria. As malaria is a widespread disease, resistance of *Plasmodium* species towards conventional treatments has driven our attention towards the cons of this therapy. Another factor that has encouraged us to review use of these conventional medicines is their toxicity and after drug adverse effects. A large repository of herbal medicines can be seen as a solution to all these problems. The review is focussed on the current scenario of malaria, its causative agents and its treatment available by conventional medicine. It also illustrates the drawbacks of conventional medicines which have led to emergence of herbal medicines to treat malaria. Recent studies have emphasised on the anti-malarial activity of herbal medicinal plants that are effective against *Plasmodium* species and have become as a potential alternative malarial drug. However, should be deduction of efficacy and toxicity of herbal anti-malarial agents should be done so that they can be used efficiently to combat malaria.

Keywords: Malaria, conventional medicine, scenario, threatening

1. Introduction

Malaria is a major public health problem which is spread throughout the world. WHO estimates 400-500 million cases of malaria throughout the world which result in approximately 1.5 – 2 million deaths each year [1]. Mosquito borne disease, malaria is caused by parasite belonging to the genus *Plasmodium* which invades red blood cell (RBC) and destroys them causing primary symptoms of malaria such as chills and fever. It is difficult to deal with the disease and overcome with it because of its complex life-cycle and transmission of the disease. Malaria has become leading cause of morbidity and mortality in tropical and sub-tropical countries. There are five *Plasmodium* species which are responsible for the life threatening malaria such as *malariae*, *ovale*, *vivax*, *knowlesi* and *falciparum*. *Plasmodium falciparum* is deadliest of all the other *Plasmodium* present (*vivax*, *malaria*, *ovule* and *knowlesi*) [2]. Malaria is predominantly infectious in infants and pregnant women [3] an Methods to deal with the disease still remain the same which is based on Insecticide Treatment and Indoor Residual spray which is approved by WHO [4, 5]. Many anti- malarial drugs used for the treatment of the disease include artemisinin, mefloquine, chloroquine but their use has led to resistance in many strains of *Plasmodium* mainly against *Plasmodium falciparum*. Other devised strategy such as Artemisinin Combinational Therapy (ACT) was used in combination with available drugs but many *Plasmodium* strains have been seen to develop resistance against them [6]. Herbal medicine plays an important role in treatment of the disease and can also prove effectively important in combating with the toxic effect caused by conventional medicines. *Artemisia annua* which is also commonly known as wormwood is normally present in various region of Asia. This tree was traditionally used medicine for fever which was also a potent source of anti-malarial activity. The objective of the review was to assess role of herbal medicinal plants which possess anti-malarial activity. Herbal medicinal plants are the greatest source for the eradication of various diseases whose treatment with conventional drugs is challenging and toxic. So, validating this approach may open gates for new and better medicines.

2. Epidemiology of Malaria

2.1 Disease burden: According to the September 2015 release of WHO, 214 million cases of malaria were reported in 2015 and malaria was also the primitive cause of 438000 deaths in sub-African region [7]. Africa continues to be home of malaria from where most of the cases are reported. In areas with high rate of transmission of malaria, children and pregnant women are at higher risk. Countries with lower GNI per capita are estimated with highest mortality rate.

2.2 Transmission: The disease is caused when infected female *Anopheles* mosquito bites that inject sporozoites in to the human. These sporozoites travel through the blood and reside into the liver cell called hepatocytes where they divide to form merozoites. Further the merozoites divide until they burst from the hepatocytes to invade red blood cells (RBC) and infect them. After several asexual cycles of sporozoites they may undergo sexual cycle and form gametocytes. Fate of these gametocyte may take two paths depending on whether they remain in the latent phase and do not harm the recipient or may be ingested by the healthy *Anopheles* mosquito from these recipient (infected humans) by biting these infected humans and convert these gametocytes into male and female gametes in the gut of the mosquito (healthy *Anopheles* mosquito) which is further mated to form zygote which develops to form oocytes. When these mosquito bites a healthy human these transfer the oocytes from the larvae of the mosquito to healthy human beings which can lead to transmission of the disease. This life cycle keeps on going and requires both human and mosquito to complete its life cycle [8]. The causative agent *Anopheles* mosquito emerges to become adult mosquitoes from the larvae formed by the egg laid by the anopheles mosquito in water. These mosquitoes then require blood to nurture these eggs. The transmission of the disease is more in the places which have longer life span of mosquitoes and they prefer to bite humans than animals. Transmission is also highly affected by the climatic conditions where the survivals of the mosquito's chances are higher. The temperate and humid region has high possibility of propagation of these mosquitoes.

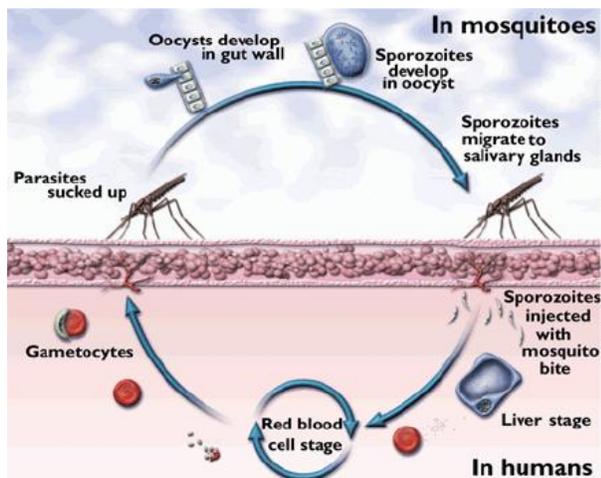


Fig 1: Life cycle of malaria (Source: <http://mtsbarama.weekly.com>)

3. Symptoms: The symptoms of malaria arises in approximately 7 days or may be more after injection of sporozoites by infected female anopheles into the human. The primary symptoms of the disease are headache, fever chills, and vomiting. Out of the strains of *Plasmodium* if *Plasmodium falciparum* is left untreated it could be life-threatening and also possess threat for anaemia, respiratory illness or cerebral malaria [9].

4. Prevention: One way to reduce malaria at community level is vector control. Vector Control Interventions if covered within specific area can prove suitable enough for the community to get protection from the disease. First step in solving this problem is to get protection from bites of the infected mosquito. In order to do so we can use long lasting insecticide treated nets and spraying insecticides.

5. Diagnosis: According to WHO, all the suspects of malaria can be diagnosed for the same through Rapid Diagnostic test (RDTs) [10] or light microscopy before any treatment.

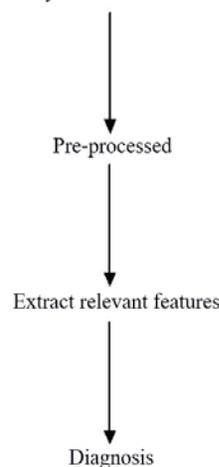
5.1 QBC Technique [11]: This technique was developed for detection and diagnosis of malaria. This involves DNA of parasite to be stained with fluorescent dye and an anticoagulant in a tube. Then the tube is centrifuged at 12,000 g for 5 min and examined with the help of epi-fluorescent microscope. Under the microscope it is seen that the nuclei of the parasite appears to be bright green while cytoplasm appears yellow-orange.

5.2 Serological Tests: Detection is based on detection of the antibodies against asexual blood stage malaria parasite. A reliable serological testing uses immunofluorescence antibody testing and is regarded as gold standard for testing of malaria serologically. The test is time consuming but is specific and highly sensitive.

5.3 Microscopy [12]: Giemsa stained blood smear slide is prepared and after capturing of these Giemsa stained blood smear images, they are loaded on to the personal computer, after that various steps are integrated which include processing of the images and neural network classifiers is implied. This leads to detection of infected erythrocytes.

5.4 Artificial Neural Network [13]

Image of infected erythrocyte and Non-infected erythrocyte were taken.



6. Traditional drugs used for treatment of malaria: Treatment through drugs involves treatment based on the species of *Plasmodium* and susceptibility to other class of parasites. Major class of antibiotics that are in use are amino quinines such as:

6.1 Chloroquine [14, 15]: This is the first line of treatment for malaria caused due to falciparum mostly in sub-Saharan Africa. These drugs inhibit detoxifying of haemoglobin breakdown product. But resistance to this medication has led to emergence of the new treatment that could battle with the disease.

6.2 Mefloquine [15]: This drug, also known as 4-quinolinemethanos, is available as an oral formulation only. It acts by inhibition of heme polymerization. Mosquitoes have acquired resistant to this drug mainly in region of Thailand. Still, this drug is prescribed to pregnant women in second half of their pregnancy.

Another class of antibiotics that are in practice to treat malaria include:

6.3 Halofantrine [15]: It is methanol derivative which is mainly related to quinine and mefloquine. *In vitro* mefloquine is more active than halofantrine. This drug is available in oral form. When these drugs are taken along with food, the absorption of these drugs increases significantly. These drugs are used for mild and moderate *P. falciparum* causing malaria. These drugs are widely available in Africa and Europe but not marketed in United States and Canada.

6.4 Artemisinin (qinghaosu) [15, 16]: Structurally unrelated to any known anti-malaria drug, artemisinin is naturally occurring lactone peroxide. It was isolated from a plant used by traditional practitioners of China mainly for treatment of fever. It is available as parental compound artemisinin as three semi synthetic derivative-

Water – soluble salt

Oil soluble compound arthemether

Oil soluble compound arteether

It is promising anti-malarial agent against *P. falciparum* strains with great speed of action. It shows promising results for short term therapy which can lead to eliminate effect such as neurological effect due to prolonged use of these anti malarial drugs.

7. Anti-malarial Drugs in Clinical Phases: There are various drugs that are under different phases of Clinical trials and targeting the life cycle of the parasite. Several drugs are also there that are effective as well as they are no-toxic but still under the trial. Some of them are illustrated below-

Table 1: Drugs under clinical phase

S. No	Anti-malarial Compound	Target site	Adverse effect		
1.	Primaquine [17]	Acts on gametocytes of all Malarial species	On	Risk of hemolysis	In G6P Deficient Patients
2.	Bulaquine [18]	Pro-drug converted into Primaquine	Into	No risk	
3.	Tafenoquine [19]	Acts on asexual Erythrocytic stages of <i>P.falciparum</i> and vivax	On	Daily dosage is needed	
4.	Spiroindalone ²⁰	Inhibit synthesis in the target parasites	Protein	Still in trials	In For Toxicity
5.	Trioxaquine ^[21]	Active form against all erythrocyte	Against	Non -toxic and Genotoxic	
6.	Epoxamicin [22]	Proteasome Inhibitor			
7.	Isoquine [23]	Avoid formation of quinine imines		Cause damage to Liver	
8.	Quinolone [24]	Inhibitor	Of		

8. Pre and Post works on Plasmodium

8.1 Pre-erythrocytic projects on Plasmodium [26]: Pre-erythrocyte subunit vaccines or whole organisms conferred immunity on *Plasmodium* which cannot imitate naturally acquired immunity but is as effective. Vaccine antigens can be taken as target candidates from pre erythrocyte stage. These candidates are further claimed by the antibodies as the

target which do not allow sporozoite invasion of hepatocytes. If complete Pre-erythrocytic vaccination occurs it leads to inactivation of the parasite before it leaves the liver leading to immunity and prevention of disease. Pre-erythrocytic vaccine when partially effective can lead to reduction in mild disease and mortality [27].

8.2 CS Protein [28, 26]: It is a protein expressed during sporozoite formation and early stages of parasitic infection. This prtein is involved in sporozoite adhesion to the hepatocytes and invasion of the hepatocytes. Conserved regions of Asparagine-Alanine-Asparagine-Proline amino acid repeat sequence can be used to raise antibodies. *P. falciparum*, B-cell epitopes are able to block sporozoite invasion of hepatocytes [26]. CS is the most popular vaccine candidates and efficacy is higher.

8.3 RTS, S/AS01E [26, 29]: This is the most advanced anti malarial candidate vaccine and most ahead project of all the malaria vaccine projects. It has been demonstrated that it has 51% efficacy in demonstrating the reduced level of clinical malaria.

8.4 Adenovirus (Ad35 vectored CS) [26, 30]: It showed modest CD4+ response but CD8+ response was low. RTS, S/AS01 with CD8 inducing CS vaccine is boosted with this combination. Non- replicating adenovirus 35 encodes for the CS protein [31].

8.5 PfSPZ [26]: It is a non–replicating malarial sporozoite vaccine which is metabolically active but challenges arise during manufacturing and scaling up of these vaccines. A Biotech Company has developed an approach administrating non-replicating, metabolically active enzyme through injection which can be thawed in liquid nitrogen for further use [32].

9. Resistance to conventional drugs urged need for herbal medicines

Hemingway *et al* [33] demonstrated that insecticide target site could either be blocked by mutation or by increasing the quantity of enzymes produced by insects. The block due to mutation can either sequester the molecules or metabolize the insecticide so that they do not function properly. One of the known causes of resistance is mutation in sodium channels that leads to resistance to DDT and Pyrethroids.

This reduces the target site sensitivity opening individual channels and leading to death or paralysis of the insects [34]. This type of resistance is phenotypic and widely seen in *An. Gambiae* species. In Africa, knock-down can play vital role in substitution such as substitution of leucine to serine at the same codon [35]. Substitution of leucine from phenylalanine also makes knock-down resistance a major reason of resistance. Nkya *et al* [36] collected agricultural samples and observed high resistance level to deltamethrin and no knock down resistance was detected. Metabolic detoxification can also result in resistance through increased enzyme activity such as esterase or glutathionic transferase. Regular use of any compound is the primary cause of resistance but information about the same is very less.

10. Potential plant and oils for prevention of malaria

10.1 Lemon eucalyptus [37] (*Corymbia citriodora*) extract: Lemon eucalyptus is also known as *Corymbia citriodora* (Myrtaceae) extracted from the leaves of lemon eucalyptus is a natural potent repellent. During screening of various plants

from Chinese traditional medicine it was found that Lemon eucalyptus essential oil comprises of 85% citronella that is widely used by cosmetic industries for its fresh smell. After hydro-distillation, the water distillate remaining of this essential oil was observed and it was seen that these were more effective at repelling mosquitoes compared to essential oil itself. But the greater disadvantages lie in the fact that they are highly volatile so though they are effective repellents but for shorter period and evaporate easily leaving user unprotected. To solve this evaporation problem, monoterpenes found in most plants came into role which contains para-methane 3, 8 diol giving higher protection at border range and for several hours.

10.2 Neem: Widely advertised natural alternative tested for repellency is Neem^[37] but EPA has not approved Neem as insect repellent. It has been reported that NEEM can be used as an alternative to N,N-Diethyl-meta-toluamide (DEET) and testing has been done against various medically important arthropods. Neem has low dermal toxicity and can also lead to skin irritation in many cases.

10.3 Lamiaceae: Essential oil from Lamiaceae^[37] (mint family that includes most culinary herbs), Poaceae (aromatic grasses) and Pinaceae (pine and cedar family), are commonly used as insect repellents. In rural areas many people use these oils either by hanging or burning them inside their homes. Now-a-days commercially available repellent also contain these oils which includes cedar oil, thyme oil, etc. Many of these oils evaporate fast and longevity is also low so to solve this problem 5% vanilla is used which is reported to repel 3 species of mosquitoes. Although these oils are highly recommended in endemic areas but using higher concentration of these oils is unsafe.

10.4 Natural oils and emulsions: Repellence against mosquitoes is shown by some oils also which work in several ways-

- 1) Evaporation and absorption are reduced by repellent actives due to presence of long chain fatty acids
- 2) High concentration fatty acid content which is repellent to mosquito.

A commercial preparation containing glycerine, lecithin, oils of coconut, geranium, vanillin and 2% soya bean oil can be used which is equivalent in repellency as to DEET. It provides around 7 hour protection. Although soya bean oil is not EPA registered but used in small amount in order to get protection because of its low dermal toxicity. Normally three oils which have been reported to be effective repellent are coconut oil, palm nut oil and andiroba oil.

11. Promising developments in plant based repellents: Plant-based repellents are in high demand by consumers due to their pleasant smell and environment friendly nature. The most important challenge lies in improving the longevity of these repellents. Some of these repellents are volatile in nature such as citronella. With the recent development of nano emulsions various formulations are undergoing research to check the increase in longevity of the plant derivative. Olfactometry demonstrated that *Ocimum forskolei* repels but does not inhibit response to human odour whereas DEET inhibits mosquito response to human odour. Another study also reveals that Citronella directly activates a cation channel that is almost equivalent to the repellent effect of pyrethrin – another plant based terpene. Pyrethrin and citronella works in

exactly opposite way as of DEET. Later analogs of pyrethrin was seen in *Tanacetum cinerariifolium* from the Dalmatian region and *Tanacetum coccineum* of Persian origin. *T. cinerariifolium* is used as an effective insecticide that is harmless to mammals. This insecticidal component comprising six esters (pyrethrin) is found in tiny oil-containing glands on the surface of the seed case.

12. Herbal drugs- A new revolution

To treat malaria new anti-malarial compounds are needed which can draw attention towards traditional medium. There are 1,277 plant species from 160 families as referred by Willcox (2008) that can be used to treat malaria^[38]. Foundation of early drugs and research are the active constituents of plant. These constituents of the plant can be isolated, identified and used for betterment of human beings. Alkaloids were the first constituents that were obtained from the plants. Many conventional drugs also have originated from plants. Modification in active constituents of plants is done to obtain the active ingredients whereas in herbal medicine no modification is done. Whole plant material is used only when the toxicity is minimal.

The major herbal plants that are used for anti-malarial activity include *Artemisia annua*, Asteraceae, which constitutes compound called artemesinin. Artemesinin is responsible for the antimalarial activity and also contains several constituents that can improve pharmacokinetic parameters^[39].

In species of *Cinchona* there are almost 7 alkaloids present which shows brief amount of anti-malarial activity. For example, totaquine which can be prepared easily from bark of the cinchona tree as an effective anti-malarial agent. Although the major side affect totaquine that it can cause vision problem and nausea.

Another medicinal plant, *Spilanthes acmella* that been authorized by National Institute of Public Health in Mali^[40] is also reported to have anti-malarial activity. *Spilanthes* remedies are usually prepared as tea substitute as seen to have highest activity^[41]. Alkylamides and flavonoids are the principle compounds that have been also found in this medicinal plant which is a local anaesthetic and known as affinin that can be used as relaxant.

Zanthoxylum chiloperon^[42] is another highly promising plant that is commonly found in the areas of Southern American. Owing to its emmenagogue and antirheumatic properties it was traditionally used to treat malaria. This compound has shown to exhibit anti-parasitic activity as well insectidal activity but, their biological activity and chemistry is still not clear.

OMARIA^[43] is a drug used in Odisha provinces for treatment of malaria. Its antimalarial activity was tested *in vitro* on *P. Falciparum* strain with chloroquine sensitive and resistant strain. Toxicity for the same was analysed by MTT assay. *Punica granalum* is used to make OMARIA. *Punica granalum* is a fruit plucked at chloroplast stage, cut and the aril is discarded. The remaining part is pounded. It is further absorbed in DMSO and serially diluted to analyze its cytotoxicity and selective index^[44]. *P. granalum* also possess anti-malarial and anti-inflammation activity^[45, 46] and the compounds responsible for this activity in *P. granalum* are still in being investigated.

13. Microbiome and Probiotics- Inexpensive technique

Microbiome is a community of microorganisms that resides in environment creating ecosystem. Human microbiome is made of pathogenic, commensal and symbiotic including fungi and viruses. Gut is basically alimentary canal or gastro-intestinal

track used to transfer food to the digestive organs. Here reside plenty of microbes that are also helpful in tremendous ways. There are number of reports that clearly states diversity in the gut microbial fauna. In case of both human as well as mosquito gut microbiome can play a significant role. There is a dynamic shift in microbiome of mosquitoes gut when they shift from aquatic to terrestrial habitat. This rapid change is generally observed when they are towards their growing phase from larvae to pupae to adults. This suggests there is a symbiotic association between host and the gut inhabitants^[47, 48, 49] which can be deduced through metagenomic profiling. This gave researchers idea that one could come out with the most unimaginable way to combat the disease. Now-a –days this is wide area of research as this can be the best to combat with the disease in most inexpensive and non-toxic way. This also drew attention of Microbiologist George Dimopoulos of John Hopkins University^[49] while he was performing an experiment with the bacteria of species *Enterobacter*. He extracted 2 microbes from the petri plate and when these microbes were sucked by the mosquito they show prevention from the *Plasmodium*. This gave him idea to feed the mosquitoes with the artificial nectar that was supplemented with *Enterobacter*, which made mosquito resistant against the the *Plasmodium*. This experiment gives us great piece of information that considering and profiling of microbiome can help us to develop a vaccine that is effective as well as inexpensive with least side effects.

14. Initiative of Organization: The Research Initiative on Traditional Antimalarial Methods (RITAM), Doctors for Life, ICIPE and the Plant Medicine Innovation Group has dedicated their energies towards the political, economic and research efforts of medicinal plants and other issues related to health and malaria. Many of these researchers believe that medicinal plants have the potential of solving the medical and societal issue of multi-drug resistance.

An organization that deserves particular mention is Action for Natural Medicine International (Anamed International). Since 1998 Anamed International has travelled to over 75 countries distributing 1,200 A. *annua* “starter-kits” (containing seeds and instructions for their use). Further activities include organizing over 100 week-long training seminars on natural medicine in 20 different countries, majority being in Africa.

PATH’s Malaria Vaccine Initiative (MVI) seeks to accelerate the development of promising malaria vaccines and ensure their availability and accessibility in the developing world. In partnership with companies, government agencies, and academic institutions, MVI is coming up with several *P. falciparum* vaccine candidates, targeting all stages of the parasite’s life cycle. As the leading killer of African children, *P. falciparum* is the primary focus of MVI’s work.

15. Conclusion: As *P. falciparum* is the most life threatening of all types of malaria causing parasite and is also resistant to all types of drugs that are mentioned in this review. *P. falciparum* predominantly attacks children and pregnant women. So, various types of medicinal plants that have anti-malarial properties should be scanned for the same which would also lead to cost effective drug synthesis. As we know side-effects related to these medicines is also less. Poor people cannot afford medicines that are prescribed for the malaria as they are costly, so herbal medication can be a boon for them. These kinds of medicinal plant can also be beneficial for other kind of disease such as cancer and other parasitic diseases. Thus, before these beneficial species of

plant become extinct they should be conserved by use of plant tissue culture and extract the substances that are helpful to combat the disease. Now- a –days various technique are possible in which one can search for the potent and effective candidate that could work as medicinal candidate. Among which one is Bio-informatics, in which we can in-silico go through its properties which may reduce time and other resources.

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