

CITRUS AND ITS BIOACTIVE COMPOUNDS: A POSSIBLE ALTERNATIVE SOURCE FOR ANTIMALARIAL DRUGS

Abstract

Malaria is a parasite disease that can be fatal and is quite common in tropical and subtropical areas of the world. Five species of plasmodium, including Plasmodium falciparum and Plasmodium vivax, are primarily responsible for the incidence of sickness. Numerous artemisinin and chloroquine-resistant plasmodium strains have recently been identified as a result of widespread medication use. Plant bioactive chemicals target many plasmodium cellular and metabolic pathways. Citrus' importance in pharmacology and medicine has long been established. The method of treating malaria, however, has not yet been examined. Thus, the ability of citrus to prevent malaria is explained.

Keywords: Citrus, Bioactive compounds, malaria, plasmodium, metabolites, medicine

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I. INTRODUCTION

Plants and products derived from plants have long been utilised in medicine. With the quick development of technology, extracting complicated plant-based compounds has aided in the creation of new medications. Mankind has always had trouble using chemicals as medicines because of their possible adverse effects. Herbal treatments, however, are less expensive and safer in contrast. Plants have remarkable medicinal potential because of the variety of bioactive substances they contain, including terpenes, phenols, coumarins, flavonoids, and alkaloids. For instance, vinka alkaloid from *Catharanthus roseus* has been used in clinical settings to treat lymphatic and haematological neoplasms since it is a potent anti-cancer agent [3]. By preventing platelet aggregation and inflammation, many phenolic chemicals found in plants, such as caffeic acid, quercetin, and apigenin, can lower the risk of cardiovascular illnesses [59]. Strong anti-diabetic properties can be found in the curcumin, berberin, and capsaicin extracted from *Curcuma longa*, *Berberis vulgaris*, and capsicum, respectively. To combat diabetes, curcumin particularly inhibits the enzyme activity of α -amylase and β -glycosidase [60].

A larger emphasis has been placed on extracting plant-based compounds to treat various parasite disorders also, particularly malaria, because plants contain a huge diversity of therapeutic compounds. Malaria is a life-threatening parasitic disease spread by bites from infected female Anopheles mosquitoes. According to the latest WHO estimates, 241 million people contracted malaria, resulting in approximately 627 thousand deaths worldwide in 2020. Malaria is known to be caused by a unicellular protozoan parasite belonging to the genus Plasmodium. Although, more than 150 different species of plasmodium infect animals, five species, namely *P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale*, and newly discovered *P. knowlesi*, cause severe Malaria in humans. *P. falciparum* and *P. vivax* are extremely common in Sub Saharan Africa, South East-Asia and the Western pacific regions of the world. In 2017, *P. falciparum* infection was responsible for about 99% of all the malaria cases in Africa [1]. *Plasmodium knowlesi* mediated malarial disease was first reported in Malaysia and then gradually observed in Thailand, Myanmar, Philippines, Vietnam, Brunei, and Cambodia [2].

The possibility of a new antimalarial drug is assessed according to a number of criteria, including novel modes of action without cross-resistance to the antimalarial drug now in use, single-dose treatments, and efficacy against both the asexual blood stages and the gametocytes that transmit the disease. Additionally, the new antimalarial drug should be effective at removing hypnozoites of *P. vivax* from the liver (anti-relapse agents) and preventing infection (chemoprotective agents). The traditional drugs follows plenty of approaches by optimising the current treatment regimens and formulations, the alteration of antimalarial medications, the screening of natural products, the isolation of molecules that reverse resistance, the use of combination chemotherapeutic strategies, and the exploitation of medications approved for other purposes are some of these methods. [63, 64]. Quinine, the first antimalarial medicine was discovered in Cinchona plants belonging to the family Rubiaceae (Fig.1.). During the twentieth century, Chloroquine, another excellent antimalarial medicine and a derivative of 4-aminoquinoline, was widely utilised (Fig.1.). However, the first chloroquine resistant parasites were identified in South-east Asia and South America. The resistance to chloroquine is due to a mutation in PfCRT gene, that code for Chloroquine resistant transporter protein, which facilitates chloroquine efflux from the

parasite's digestive vacuole [5]. Currently, the most preferred choice of drug against malaria is artemisinin. Artemisinin is a sesquiterpene lactone endoperoxide isolated from Chinese wormwood *Artemisia annua*, is highly effective against Chloroquine and sulphadoxine-pyrimethamine resistant plasmodium [6]. Generally, Artemisinin is administered in conjugation with other medications, however recent data suggests that artemisinin-resistant Plasmodium species may be evolving [7]. Using CRISPR-Cas9 genome editing tools, transgenic mosquitoes with low blood-sucking propensity, egg hatchability, and reduced longevity have been engineered [4]. However, utilising genetically engineered mosquitoes raises various ethical concerns, thus widespread use is still restricted to field experiments in several countries. Due to these restrictions, we searched for other molecules that could function as powerful antimalarial agents. Many different bioactive chemicals found in plants can be used to create novel drugs. For instance, Quinine (Fig.1.) is an alkaloid collected from the chinchona plant, whereas artemisinin is a sesquiterpene isolated from *Artemisia annua*. Naphthoisoquinolines a type of alkaloid isolated from various plants including Acistrocladaceae family have shown to exhibit anti-malarial activities against *P. falciparum* strains [8]. Plant based flavonoids, terpenes and phenolic compounds are also active in treating malaria. Limonoids, for example, isolated from *Carpaguanensis* show dose dependent suppression of *Plasmodium berghei* NK65 strain [9].

Compared to other plants, citrus plants have a wide variety of the bioactive substances that were previously addressed. The fruits, which are consumed all over the world, contain a variety of secondary metabolites, including coumarins, terpenoids, limonoids, alkaloids, and flavonoids. These bioactive substances' existence has confirmed their function in the anticancer, anti-inflammatory, antifungal, and antibacterial characteristics. Ideally, citrus plants have previously shown the ability to efficiently heal parasite infections. *Citrus sinensis* essential oil was discovered to be the most efficacious in an in vitro setting against *Leishmania amazonensis* [61]. Sinesetin and Nobiletin extracted from different citrus cultivars possesses exceptional antitrypanosomal activity [62]. Therefore, it may be concluded that citrus plants will serve as a substitute antimalarial food and medicine source due to the bioactive chemicals they contain. In this review, we have examined the existing literature and provided support for the claims we have stated.

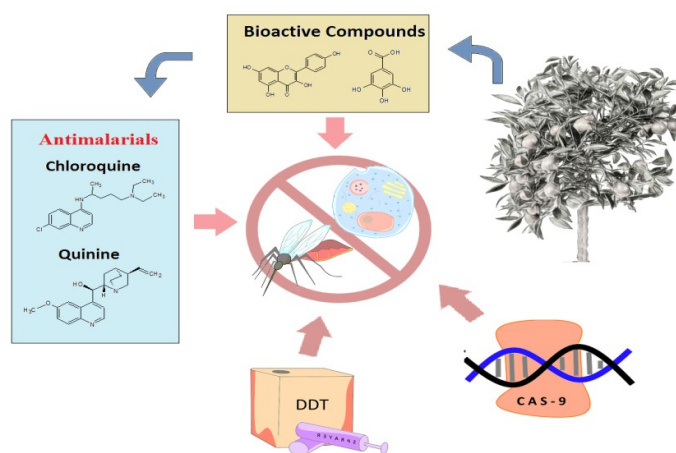


Figure 1: Diagram showing different methods used to control Malarial incidence

II. IMPORTANT BIOACTIVE COMPOUNDS IN CITRUS

Citrus plants are a rich source of bioactive substances, the majority of which are secondary metabolites such as Phenolics, Terpenoids, Flavonoids, Coumarins and Alkaloids. Citrus holds a significant position in the pharmaceutical and medical industries thanks to the existence of these bioactive compounds.

- 1. Flavonoids:** Flavonoids are low molecular weight phenolic compounds that serve to draw pollinators and gives flowers and fruits their perfume and colour. Flavonoids are highly effective antioxidant and chelating agent because they contain numerous hydroxyl groups. Two phenyl (A and B) and a heterocyclic rings (C) make up the fundamental structure of a flavonoid (**Fig.2.**). Flavonoids are split into eight categories based on the attachment of these rings, including Flavones, flavonols, Flavanones, catechins, anthocyanins and chalcones [10]. Out of these subtypes most of the citrus fruits contains high number of flavones and flavanones. Neohesperinoids and rutinoides account for majority of the flavones in tropical citrus whereas apigenin (**Fig.2.**), phloretin (**Fig.2.**), hesperetin and naringenin are types of flavonoids that occur in moderate amount [11].

Citrus flavonoids can be extracted from a variety of fruit sections, although according to numerous studies, citrus fruit peel is considered to be the primary source of flavonoids [12]. For example, HPLC analysis of *Citrus reticulata* peel extracts led to the isolation of naringin, hesperetin, hesperidin, rutin and catechin in excessive amount [13]. The peel extract of *Citrus aurantium* also contains significant quantity of naringenin and apigenin [51]. Additionally, citrus fruit juice is well known for its flavonoid content. Apigenin and eriocitrin (5,7,3',4'-tetrahydroxyflavone 7- β -rutinoside) is commonly found in lemon, orange, grapefruit and bergamot varieties of citrus. Whereas limocitrin glucoside and diosmetin flavonoids are only found in lemons [52]. It has already been established that flavonoids isolated from plants are beneficial in treating malaria. Nymphaeol and solophenol flavonoids isolated from *Macaranga tanarium* leaves have showed satisfactory antiplasmodial activity [14]. 1-(2,4-dihydroxy phenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3pentenyl)-2H-1-benzopyran-5-yl]-1-propanone designated as Compound 1 by Hidayati[15] was isolated from *Artocarpusaltilis* leaves. The flavonoids IC₅₀ value for *Plasmodium falciparum* growth inhibition during antiplasmodial screening was 1.05 μ M.

- 2. Alkaloids:** Alkaloids are natural chemical containing Nitrogen and are most frequently found in flowering plant species. Alkaloids (**Fig.2.**) may or may not have heterocyclic rings. True alkaloids and protoalkaloids are the two categories for alkaloids derived from amino acids. Pseudoalkaloids are instead derived from precursors of amino acid and are related to amino acid formation pathway [16]. Some typical examples of true alkaloids include quinine, morphine and, cocaine. Arcidone alkaloids are pretty prevalent in the Rutaceae family and have also been proven to have antimalarial effects. Normelicopidine, a type of arcidone alkaloid isolated from *Zanthoxylum simullansm* displayed effective antimalarial activity against chloroquine-sensitive (3D7) and (Dd2) strains of *Plasmodium falciparum* [17]. Many different alkaloids have been extracted from citrus plants as well. DCM fractionation of *C. aurantium* showed the presence of six arcidone alkaloids i.e. citrussinine-I, 5-hydroxynoracronycine, glycofolinine, natsucitrin-I

and citracridone-III [18]. Octopamine, Synephrine and Tyramine (**Fig.2.**) have also been isolated from wild citrus hybrid plants using the HPLC-UV detection method [19].

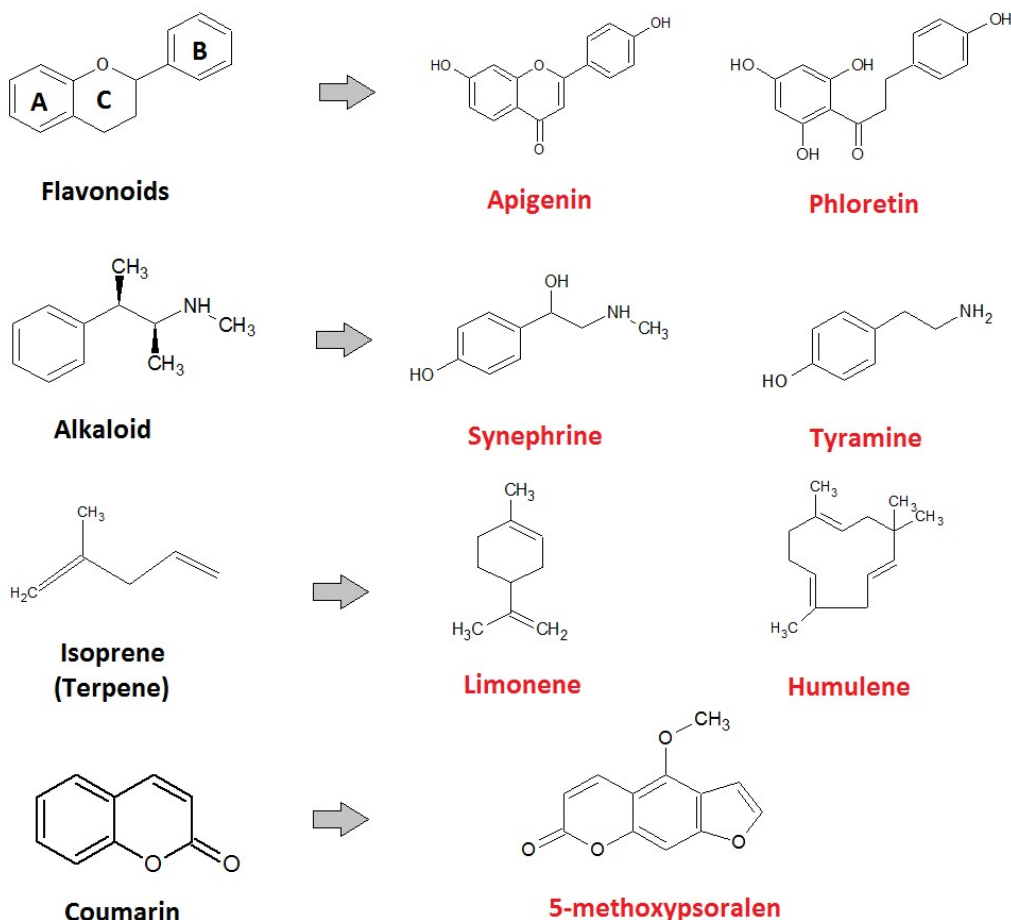


Figure 2: Diagram showing the structure of common metabolites found in citrus plants.

- 3. Terpenes:** Terpenes are the major constituents of citrus essential oil. Terpenes are generally made up of either single or multiple Isoprene units with molecular formula C_5H_8 (**Fig.2.**). Based on the number of isoprene units, terpenes can be divided into mono (eg. Cineole, linalool, citronellol, cymene, carane, thujone), di (eg. terpineol, phytol, taxol, carnosic acid), tri (eg. Sitosterol, stigmasterol, amyirin) and sesquiterpenes (eg. humulene, cadinene, germacrene, curcumene) [20]. Terpene Hydrocarbons (**Fig.2.**) such as Limonene, humulene, ocimene, pinene, sabinine myrcene accounts for majority of the terpenes found in citrus essential oil followed by terpene alcohols and keto-terpenes [21]. Numerous terpenes have been found to be present in *Citrus aurantium* peel essential oil (EO). Benayad [51] reported the presence of monoterpene hydrocarbons such as limonene, myrcene and linalool in excessive amount. However, Jha [54] discovered β -piene, D-limonene, eucalyptol and terpineol in *Citrus aurantium* flower EO. Different *Citrus paradisi* varieties are said to contain high amounts of Limonene in their peel and juice extract. Additionally, a moderate amount of Ocimene, carene and carveol can also

be found in *Citrus paradisi* varieties [53]. Some studies have reported to found Citronellol (28.26%) an acyclic monoterpene to be present in highest amount followed by β -caryophyllene (16.89%) and spathulenol (9.32%) in some citrus varieties [22]. Limonene (**Fig.2.**) a cyclic monoterpene hydrocarbon may occur in concentration as high as 58% - 87.84% in essential oil of mousami, grapefruit, mandarin and malta [23]. The presence of high number of terpenes in citrus essential oil makes it an important contender for antimalarial activity. Nerolidol a sesquiterpene alcohol for example has the ability to inhibit *Plasmodium berghei* both in vitro and in vivo. Nerolidol at dose of 1000mg/kg decreased parasitemia in infected mice by >99% (oral administration) and >80% (inhalation) [24].

- 4. Coumarins:** Coumarins (**Fig.2.**) are heterocyclic compound with chemical formula $C_6H_6O_2$ and is made up of a pyrone ring united to a benzene nucleus (1,2-benzopyrones). Based on the chemical structure, naturally occurring coumarins are divided into seven different types. These types include simple coumarins, pyranocoumarins, bicoumarins, phenylcoumarins, furanocoumarins and dihydrofuranocoumarins. Citrus plants contain abundant subtypes of coumarins which are usually associated with the defence mechanism of plants. Pumelos, citrons and papedas have been found to synthesize large amount of furanocoumarin and coumarins, but mandarins lack both in sufficient proportion [25]. 5-geranyloxy-7-methoxycoumarin, 5,7-dimethoxycoumarin, 5,8-dimethoxypsoralen, 5-methoxypsoralen and 5-geranoxypsoralen (**Fig.2.**) are key coumarins isolated from *Citrus aurantifolia* and *Citrus latifolia*[26].

III.MECHANISM OF ACTION OF BIOACTIVE COMPOUNDS AGAINST PLASMODIUM

It has already been established that terpenes have anti-plasmodial properties. Terpenes unquestionably targets different cellular elements of the plasmodium. Non-artemisinin based sesquiterpene lactones such as parthenin and parthenolide prevent parasites male gamete exflagellation and ookinete to oocyst transition. In plasmodium, male gamete exflagellation creates male gametes from single parental cell and necessitates rapid cell division and flagellar beating. The generation of male gametes is inhibited by parthenin and parthenolide, possibly by interfering with DNA replication or by producing reactive oxygen species [27]. Terpenes have also demonstrated hypolipidemic effect by lowering the level of triglycerids, LDL-cholesterol and phospholipids in *Plasmodium berghei* infected erythrocytes. This potentially regulates the growth of plasmodium in erythrocytes [28]. The apicoplast present in Plasmodium is a key site for the biosynthesis of Isoprenoids. These isoprenoids in *P.falciparum* are used for the synthesis of Vitamin E and dolichols. Linalool, Farnesol and nerolidol are potent inhibitor of dolichol biosynthesis in trophozoite and schizont stage of *Plasmodium falciparum* [29].

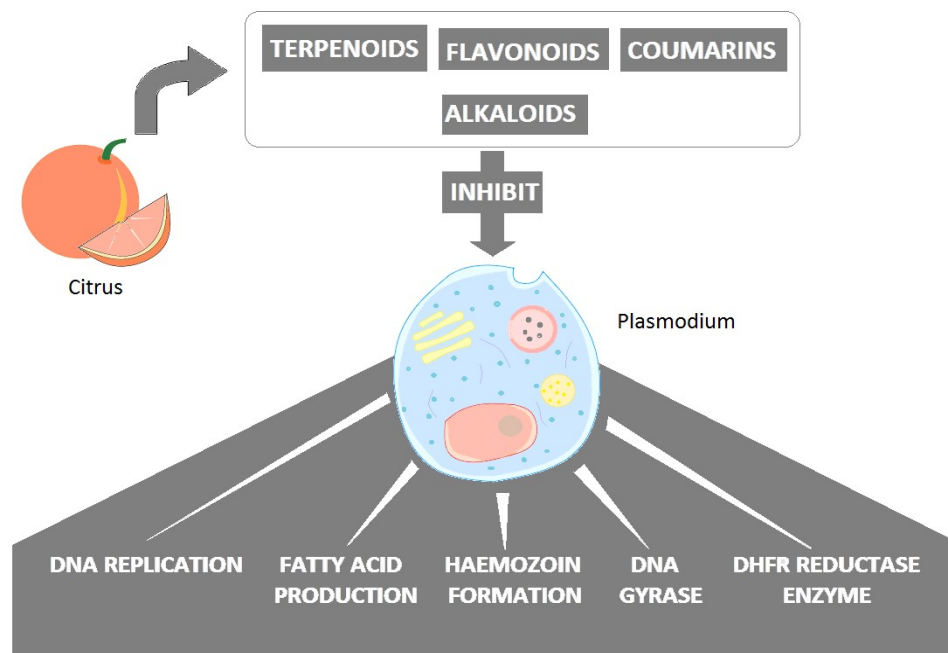


Figure 3: A simplified diagram showing the mode of action of citrus metabolites against plasmodium.

The involvement of flavonoids in targeting the plasmodium's fatty acid production pathway has been supported by some earlier studies. Flavonoids target the Fab G, Fab Z and Fab I enzymes that are crucial for plasmodial fatty acid production. The inhibition depends on complexity of the flavonoids and the presence of hydroxyl group. Polyhydroxylated flavonoids were 10 times more effective in inhibiting FabG, FabZ and FabI [30]. Additionally, flavonoids such as myricetin and fistein targets key cystein protease enzymes Falcipain-2 and plasmepsin-II which are involved in breakdown of host haemoglobin [32]. A novel flavonoid glycoside, pinocembrin-7-O- β -D- glucopyranoside has been reported to inhibit Dihydrofolate-reductase thymidylate synthase (DHFR-TS) enzyme. This enzyme in *Plasmodium falciparum* is responsible for the production of thymidylate and folate, both of which are required for DNA synthesis [31].

Many antimalarial drugs, including chloroquine, work against plasmodium by obstructing with the process of haemozoin formation. Haemoglobin degradation by malaria parasite releases free heme, which is toxic to the parasite itself. For survival, the parasite transforms the heme into hemozoin, a biocrystal malarial pigment [33]. 4-nerolidylcatechol a sesquiterpene and its derivatives have been proven to prevent the formation of hemozoin at various concentrations (Silva et al. 2015). Flavonoids with increased methoxylation (Pentamethoxyquercetin) patterns are extremely effective in forming heme-flavonoid adducts and may be the cause behind its antiplasmodial efficacy [34]. Silymarin, another polyphenolic flavonoid forms heme-silymarin complex which destabilises the cell membrane of the parasite. Furthermore, the compound can inhibit polymerisation and degradation of heme [35]. According to molecular docking studies, chalcones is a key bioactive flavonoid that targets the *Plasmodium falciparum* DHFR-TS enzyme. A derivative of the chalcone

designated 3b, used in the investigation show binding interaction with the 1J3I *Pf*DHFR-TS proteins Ala16, Ile164, Phe58 and Tyr170 residues [36].

Recent research has revealed that DNA gyrase of plasmodium can be a novel target of several antiparasitic compounds. Coumarins which are abundant in rutaceae family exert antimalarial activity by hampering the function of DNA gyrase. This leads to the formation and accumulation of single stranded DNA breaks [37]. In addition to that, coumarins can regulate hemozoin formation and DNA interaction. The IC₅₀ values have shown that coumarins are just as effective as chloroquine at preventing parasite from producing hemozoin [38].

IV. CITRUS AS A SOURCE TO FIGHT MALARIA

Citrus is one of the many plants whose antimalarial qualities have been studied over time. Citrus is a significant choice to access its potential in anticancer, anti-inflammatory, antiparasitic, antibacterial and antioxidant roles due to the presence of a variety of secondary metabolites. Citrus is a relatively new antimalarial agent thus; more attention needs to be paid to it. Extracts of citrus plant parts as well as essential oil can be used as a malaria repellent or as a drug/food source directly to cure malaria. Below we have thoroughly discussed the potential of citrus plants as an antimalarial agent.

- 1. Anti-Plasmodial Activity of Citrus:** Because they contain a wide range of important chemicals, citrus limon fruit and fruit parts are traditionally utilised as medicine in many parts of the world. Citrus limon leaf, fruit, and root extracts were found to be highly effective against plasmodium in numerous in vitro and in vivo experiments [39][40][41][42]. The effectiveness of acetone extract and *Citrus limon* leaf powder against *Plasmodium berghei* Anka-infected mice was investigated by Bonkian and his colleagues [39]. At a dose of 250 mg/kg body weight, the acetone extract was shown to be efficient and reduced the parasitaemia by almost 75%. However, it was discovered that the leaf decoction proved useless at treating malaria. *Citrus limon* fruit aqueous decoction also showed low parasitic suppression of plasmodium compared to negative infected control groups. However, when taken with conventional antimalarial medications, lemon decoction had the strongest effect against *Plasmodium berghei* [40]. A viable option for usage as an antiplasmodial agent is to combine the therapies of *Citrus limon* and *Carica papaya*. The invitro testing of this combination on chloroquine sensitive D10 strain produced an outstanding IC₅₀ value of 0.83 µg/ml. *Citrus limon* administration alone had a lower level of effectiveness. The best antiplasmodial activity was demonstrated by dichloromethane extract of *Citrus limon* with IC₅₀ value of 5 µg/ml [41]. Additionally, it was shown that the dichloromethane extract of *Citrus limon* root was effective in reducing malaria brought on by both chloroquine-sensitive and multi-drug resistant strains of *Plasmodium falciparum*. [58].

Table 1: Antiplasmodial activity of different Citrus plant extracts.

Sl. No	Citrus plant	Plant part	Extract	Test	Plasmodial species	Reference
1.	<i>Citrus limon</i>	Leaf	Acetone	In vivo	<i>Plasmodium berghei</i>	Bonkian et al. 2019
2.	<i>Citrus limon</i>	Fruit	Aqueous Decoction	In vivo	<i>Plasmodium berghei</i>	Shija et al. 2020
3.	<i>Citrus limon</i>	Leaf	Dichloromethane	In vitro	<i>Plasmodium falciparum</i>	Melariri et al. 2012
4.	<i>Citrus limon</i> + <i>Citrus paradisi</i>	Fruit	Fruit juice	In vivo	<i>Plasmodium berghei</i> ^{chl}	Elom et al. 2021
5.	<i>Citrus limon</i>	Root	Dichloromethane	In vitro	<i>Plasmodium falciparum</i>	Guya et al. 2022
6.	<i>Citrus arantifolia</i>	Fruit/fruit	Aqueous-ethanolic	In vivo	<i>Plasmodimberghei</i> ^{chl}	Odediran et al. 2020
7.	<i>Citrus aurantifolia</i>	Leaf	Ethanolic	In vivo	<i>Plasmodimberghei</i>	Ettebong et al. 2019
8.	<i>Citrus aurantifolia</i>	Flower	Methanolic	In vitro	<i>Plasmodium falciparum</i> ^{chl}	Bapna et al. 2017
9.	<i>Citrus maxima</i>	Fruit peel	Methanolic	In vivo	<i>Plasmodium berghei</i>	Ihekwereme et al. 2017
10.	<i>Citrus maxima</i>	-	Hydro-alcoholic	In vitro	<i>Plasmodium falciparum</i> ^{chl}	Gogoi et al. 2021
11.	<i>Citrus sinensis</i>	Peel	Ethanolic	In vivo	<i>Plasmodium berghei</i>	Kristhien et al. 2019
12.	<i>Citrus paradisi</i>	Leaves	Ethanolic	In vivo	<i>Plasmodium berghei</i>	Kristhien et al. 2019
13.	<i>Citrus senensis</i>	Stem	Ethanolic/aqueous	In vivo	<i>Plasmodium berghei</i>	Chinwuba et al. 2015
14.	<i>Citrus aurantium</i>	Flower	EO	In vitro	<i>Plasmodium falciparum</i>	Jha et al. 2022
15.	<i>Citrus paradisi</i>	Root	EO	In vitro	<i>Plasmodium falciparum</i>	Ogunjinmi et al. 2017

Combined therapy of *Citrus limon* and *Citrus paradisi* fruit juice extract is also found to be effective in inhibiting the growth of chloroquine resistant *Plasmodium berghei* ANKA. When administered without combination, *C. limon* extracts were less powerful in treating disease compared to artemisinin combined therapy (ACT) whereas *C. paradisi* were least effective [42].

Extracts of different parts of *Citrus aurantifolia* were evaluated against *Plasmodimberghei* infected mice. The fruit extract of *Citrus auratifolia* were found to be more active in chemosuppressive, prophylactic and curative model. The percentage reduction in parasitemia for chemosuppressive mode was found to be 92.9±1.2 [44]. Antiplasmodial activity of methanolic extract of *Citrus aurantifolia* leaf showed 77.66%

chemosuppression in suppressive test conducted in mice. However, in Repository and curative tests low doses of the extract demonstrated better activity than middle or high doses. The median lethal dose was calculated to be $3280 \text{ mg/kg} \pm 0.01$ and is safe for oral use [45]. Chloroquine resistant *Plasmodium falciparum* 3D7 strain poses a considerable threat. According to some recent studies the 3D7 strain was effectively inhibited by methanolic extract of *Citrus aurantifolia*. The aqueous extract in comparison did not pose any considerable threat to the protozoan strain. [46].

Methanolic extract of *Citrus maxima* peel showed promising antimalarial properties against *P. berghei*. Mice injected with malaria were administered with 125, 250 and 500 mg/kg/day for 7 consecutive days. At 125mg/kg/day 89% of the parasites were cleared by day 7 whereas for 500mg/kg, 95.87% of the parasites were effectively eliminated [47]. The hydro-alcoholic extract of *Citrus maxima* demonstrated its effectiveness in inhibiting the growth of chloroquine sensitive (3D7) and Chloroquine resistant (RLK-9) strains of *Plasmodium falciparum*. The IC₅₀ values of *Citrus maxima* against 3D7 strains ($3.41 \pm 0.31 \mu\text{g/ml}$) and RLK-9 strains ($4.45 \pm 0.10 \mu\text{g/ml}$) were found to be better than the IC₅₀ values *A. nilagirica* alcoholic extracts against Plasmodium strains [48].

In a comparative analysis between Ethanol extracts of *Citrus sinensis*, *Citrus paradisi* and *Psidium guajava*, highest anti-plasmodial activity was shown by *Psidium guajava* with 99.12% inhibition of parasitemia. *Citrus paradisi* was moderately active with 98.84% inhibition of parasitemia at 1000mg/kg concentration. *Citrus sinensis* was found to be least effective amongst the three [49]. Earlier research on *Citrus sinensis* effectiveness against *Plasmodium berghei* produced some intriguing findings. The effectiveness of stems aqueous extract was found to be lowest, while the ethanolic extract of the stem was found to be powerful enough to suppress 30% to 50% of parasitaemia [56].

Essential oil from citrus can be extracted from various parts. However, the chemical makeup of the oil causes the effectiveness to vary. Root, fruit peel and stem essential oil of *Citrus paradisi* exhibit varying degree of effectiveness against malaria. The fruit peel oil with limonene and α -myrcene as major component showed no significant activity against *Plasmodium falciparum* strain. The root oil with IC₅₀ value of $22.2 \mu\text{g/mL}$ was somewhat effective against plasmodium parasites. Activity of stem essential oil was also not found to be satisfactory in arresting plasmodium growth [43]. EO extracted from *Citrus aurantium* flowers have shown antiplasmodial activity against drug sensitive and drug resistant varieties of *Plasmodium falciparum*. The IC₅₀ values have shown that EO of flower provides similar usefulness against drug sensitive strains when compared to that of standard quinine drug. However, against drug resistant strains the effectiveness of EO was found to be $0.97 \mu\text{g/ml}$ [54].

- 2. Citrus as an Antilarval/Mosquito Repellent:** Although it wasn't the main goal of this review, we did want to shed some light on the topic of citrus potential as a mosquito deterrent. Citrus is less toxic than chemical alternatives, making its use as a mosquito repellent a significant discovery. *Citrus limon*, *Citrus sinensis*, *Citrus reticulata* and *Citrus aurantifolia* all have effective anti-larval property [55] [56]. Aqueous extract of *Citrus limon* has shown promising results in in-vitro tests against *Anopheles gambiae* larvae and can be used to control free floating larvae found in stagnant water [56]. In

another study, aqueous extract of *Citrus sinensis* peel showed dose dependent inhibition with percentage mortality ranging from 65% to 100% when tested against mosquito larvae. This could be a reasonable and eco-friendly alternative to protect families from malarial attack [50]. *Anopheles stephensi* and *Culex quinquefasciatus* are two mosquito species that can be controlled using the essential oils of *Citrus limetta*, *Citrus reticulata* and *Citrus limon* leaves [57].

V. DECLARATION

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- 2. Funding:** No funding received in making this manuscript
- 3. Authors Contribution:** Authors contributed equally in making the manuscript.

VI. DATA AVAILABILITY

No data is available in this manuscript.

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