Research and Reviews: Journal of Pharmacognosy and Phytochemistry
e-ISSN: 2321-6182
p-ISSN: 2347-2332

A Review on Anti-Malarial Activities of Selected Plant Species from the Rutaceae Family

Lawal IO* and Olagoke TH
Biomedicinal Research Centre, Forestry Research Institute of Nigeria, Ibadan, Nigeria

Research Article

Received: 15/06/2016
Accepted: 27/09/2016
Published: 07/10/2016

***For Correspondence**

Ibrahim Lawal, Biomedicinal Research Centre, Forestry Research Institute of Nigeria, Ibadan, Nigeria

E-mail: Ibroodula@yahoo.com

Keywords: Rutaceae, Plasmodium falciparum, anti-malaria, phyto-chemicals

ABSTRACT

Advancement in phytochemical studies in medicinal plants has really helped in the production of helpful medicinal drugs. This review is aimed at investigating the anti-malarial properties of herbal plant extract so as to complement the effort to find a better and more efficient way of eradicating malaria from African countries. *Clausena anisata*, *Zanthoxylum chalybeum* and *Toddalia asiatica* were reviewed. The extract from *C. anisata* was found to possess antimalarial activities which are due to some secondary metabolite available in the plants such as alkaloids, flavonoids, monoterpenes and triterpenoids. This review shows that organic solvents of the leaf extracts of selected species contains secondary metabolite compounds with antiplasmodial activity as well as the root bark which has also been reported to have significant effect on *P. falciparum*. This review shed more light on the significance of Rutaceae family and its antimalarial properties.

INTRODUCTION

Malaria is caused by a parasite called *Plasmodium*, which is transmitted via bites of infected mosquitoes. The parasites multiply in the liver of the human body and thereafter infect the red blood cells. Between 10-15 days after a mosquito bite, malaria symptoms such as fever, headache, and vomiting begin to manifest in an infected person. Untreated malaria disrupts the supply of blood to vital organs which makes it a life-threatening parasite. It is suitably noted that in many parts of the world the parasites have developed resistance to a number of malaria medicines. The species *P. knowlesi* rarely causes disease in humans. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests [1]. Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity [2].

The use of traditional medicinal plants to fight disease has been ongoing for centuries. With the resistance of *Plasmodium* to some drugs, countries and pharmaceutical companies have turned to traditional plants for the cure and possible eradication of malaria. Various plant species have been said to cure malaria, these plants are made to undergo some processes (mostly boiling with water) in order to extract the components that combats *Plasmodium*. The boiling process might not be an accurate means for the extraction of certain plants, however, modern science has made it possible for proper extraction and thorough research on how these phytochemicals can be extracted and used to combat and hopefully finally eradicate malaria. The Rutaceae family is a family of flowering plants, usually placed in the order Sapindales. Species of the family generally have flowers that divide into four or five parts, usually with strong scents. They range in form and size and can be found as herbs, shrubs and small trees [3].

Plants investigated

C. anisata

C. anisata benth (Horsewood, maggot killer (En). Mjavikali (Sw)), is a tropical shrub or tree growing up to ten meters in height in and on the margins of evergreen forests [4]. Different parts (stem bark, roots, and leaves) of this plant are widely used in traditional medicine to treat many diseases. Traditional healers in Tanzania use *C. anisata* against oral candidiasis and fungal infections of the skin [5]. Whereas, in the Temeke district (Daressalam, Tanzania), *C. anisata* is used against epilepsy and as an anticonvulsant [5]. In some parts of Africa and in the Philippines, the burning of fresh leaves is utilized to repel mosquitoes [6]. Previous phytochemical investigations on this taxon yielded mostly carbazole alkaloids [7,8], coumarins [9,10] and limonoids [11].

e-ISSN: 2321-6182
p-ISSN: 2347-2332

Z. chalybeum Engl.

Z. chalybeum Engl. Var. chalybeum (Knob wood) is a spiny deciduous shrub or tree up to 12 m high, with a rounded but open crown. It has compound leaves consisting usually of 3 to 5 pairs of shiny leaflets plus a terminal leaflet, with a strong citrus smell when crushed. The trunk is characterized with large, conical, woody knobs with sharp thorns. The fruit is spherical, about 5 mm in diameter, reddish-brown, splitting to allow the shiny black seeds to protrude [12].

Z. chalybeum is used throughout Eastern and Southern Africa for its aromatic leaves, shoots and fruits, which are used to make a variety of tea-like beverages and decoctions. It is also widely popular as a medicinal plant for humans and animals and is sometimes over-exploited for these properties [13,14]. Its potential antiplasmodial and anti-trypanosomal properties are currently being investigated [15,16]. *Z. chalybeum* also provides good firewood and hard durable timber [12]. *Z. chalybeum* is native to Eastern and Southern Africa (Burundi, Democratic Republic of Congo, Ethiopia, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, Somalia, South Africa, Swaziland, Tanzania, Uganda, Zambia, and Zimbabwe). It can be found at medium and low altitudes, from sea level to 1600 m, under 750 mm to 1500 mm annual rainfall. It grows in dry woodland or grassland, often on termite mounds [12].

T. asiatica (Orange climber)

Toddalia is a monotypic genus [17] of flowering plants in the citrus family containing the single species *T. asiatica*, which is known by the English common name Orange climber. This is a liana with woody, corky, thorny stems that climb on trees, reaching up to 10 m in length. It has shiny green citrus-scented leaves, yellow-green flowers, and orange fruits about half a cm wide that taste like orange peel. The seeds are dispersed by birds and monkeys that eat the fruits. It is native to many countries in Africa and Asia. Examples of these countries include South Africa where in Afrikaans it is called Ranklemoentjie, and in Venda, Gwambadzi. It is very popular among the Kikuyus of Central Kenya, where it is known as mururue [18]. It grows in forested riparian habitat with high rainfall. The destruction of forest habitat in Africa threatens the species' survival [19]. The plant is used medicinally by many African peoples, including the Maasai, who use it for malaria [20], cough, and influenza. The roots contain coumarins that have antiplasmodial activity [21].

RESEARCH FOCUS AND JUSTIFICATION

Malaria is still one of the greatest global public health problems especially in sub Saharan Africa. This can be partly attributed to the development of resistance by malaria parasites to most of the established anti-malarial drugs such as chloroquine, sulphadoxine/pyrimethamine and amodiaquine [22]. Currently, fixed dose artemisinin-based combination therapies are being used as first-line treatment of uncomplicated falciparum African. Unfortunately, recent reports indicate a decline in efficacy of artemisinin derivatives. New classes of anti-malarial agents are therefore urgently needed given that the resistance of the parasite is likely to eventually compromise the efficacy of currently available anti-malarial drugs. Identification of lead anti-malarial agents from medicinal plants could boost the search.

This study is aimed at investigating the anti-malarial properties of herbal plant extract so as to find a better and more efficient way of totally eradicating malaria from Africa and other affected countries.

Anti-Malarial Activities

C. anisata

Alkaloids are one of the major classes of compounds possessing anti-malarial activity. One of the oldest and most important anti-malarial drugs, quinine, belongs to this class of compounds and is still relevant. Pure alkaloids rank among the most efficient and therapeutically significant plant compounds [23]. The bark extracts of *C. anisata* has high safety levels (LD50=3514 mg/kg), considerable suppressive effects, and significant ($P<0.001$) prophylaxis against *P. berghei* in mice. This phenomena, together with other pharmacological effects observed in other parts of the plant, such as anti-diabetic, anti-microbial, parasiticidal and spasmolytic properties observed in other experiments makes *C. anisata* a better anti malaria than other species with anti-malarial potential only [24]. This also partly explains the high survival periods of the *P. berghei* challenged mice noted after treatment with *C. anisata* extracts despite the exhibited minimal curative effect established during infection test.

Noedi et al. [25] suggested that crude plant extracts tended to have better 59 plasmodistatic than plasmodicidal effects because some unpurified bioactive principles may require initial conversions and the time lag allows for parasite proliferation. Moreover, the active components might not be present in enough concentrations to effect rapid clearance of target organisms.

Jude et al. [26] in their work investigated that median lethal dose (LD50) was determined to be (393.70-25.64) mg/kg and the extract was relatively safe. The anti plasmodial properties of the extract and its fractions were investigated using standard models. The extract and its fractions exerted significant reduction of parasitaemia in prophylactic, suppressive and curative models in a dose-dependent fashion. These activities could be attributed to some secondary metabolites of this plant which have been reported to have antiplasmodial activity with varying mechanisms of action. Among these metabolites are alkaloids, flavonoids and triterpenoids such as limonoids and quassinoids [27]. These compounds (alkaloids, flavonoids, monoterpenes and

e-ISSN: 2321-6182
p-ISSN: 2347-2332

triterpenoids) present in this plant extract may in part have contributed to the plasmocidal activity of this extract and therefore explained the mechanism of antiplasmodial effect of the extract and its fractions. The extract significantly reduced acetic acid-induced writhing, formalin-induced hind paw licking as well as delayed the reaction time of animals (mice) to thermally induced pain. Acetic acid causes inflammatory pain by inducing capillary permeability and in part through local peritoneal receptors from peritoneal fluid concentration of PGE2 and PGF2 [27]. The results of this study support the ethno botanical use of the plant in the treatment of febrile illnesses, malaria and pains. Further investigation is being advocated especially in elucidating cellular mechanisms and establishing structural components of the active ingredients with a view of standardizing them.

Beatrice et al. [28] investigated the *in vivo* anti-malarial and acute toxicity properties of hexane and chloroform extracts from *C. anisata* (Wild) enth. Two extracts were tested at 3 different dose levels for their *in vivo* anti-malarial properties and also for their acute toxicity in mice. The chloroform extract at 500 mg/kg/day exhibited promising anti-malarial activity in both suppressive and prophylactic tests. In the curative test, the same dose level was associated with an MST that was significantly longer than that of the untreated group though incomparable to that of chloroquine. The hexane extract at 500 mg/kg/day had moderate to low suppressive and prophylactic properties. In the curative test, life span of mice was also not significantly different from that of untreated group. This lower activity may probably be due to the fact that hexane extract mainly consists of fats and oils that are often not biologically active. The chloroform extract displayed some mild oral acute toxicity. Coumarins like chalepin isolated from *C. anisata* are reported to be toxic with an intraperitoneal LD50 of 100 mg/kg in rats [29]. Coumarins are also reported to be mutagenic [30] while carbazole alkaloids, the major constituents of *C. anisata*, are reported to be cytotoxic [31]. These compounds may have contributed to the observed oral mild acute toxicity.

The results of this study indicate that the chloroform extract of the *C. anisata* stem bark possesses anti-malarial activity. Further studies are required to determine if the anti-malarial activity of *C. anisata* is attributable to the alkaloid and coumarin constituents. Meanwhile, the results partly corroborate claims made in traditional medicine of the anti-malarial efficacy of this plant.

Z. chalybeum

Godfrey et al. [32] investigated the antiplasmodial activity of the ether and methanol extracts of *Z. chalybeum* using chloroquine diphosphate as positive control and the culture medium as negative control. The results show that ether extract had EC50 value of 13.39 (10.82-16.59) µg/ml at 95% CI; methanol extract had EC50 value of 8.10 (5.89-11.12) µg/ml and choloroquine diphosphate had EC50 value of 25.33 (17.07-37.60) µg/ml. The observed low effective concentration (EC50) of methanol leaf extract of *Z. chalybeum* required to suppress 50% of *P. falciparum* schizonts per 200 WBC count as compared to the ether extract and chloroquine disphosphate as control, could have been due to the various secondary phytochemical compounds that have been isolated from the leaf extract and the results show similar activity to previous studies done on the root-bark of the herb [33,34]. These compounds include the flavonoids, alkaloids, coumarins, amides, flavonoids, terpenes and sterols, sesquiterpene lactones and saponins [32,35]. The most notable secondary metabolites from various species of plants that have been reported to have antiplasmodial activity are alkaloids, steroids and triterpenoids and sesquiterpene lactones [32,33,36]. Even the currently used anti-malarial drugs in the conventional medicine are derived from medicinal herbs such as quinine, a major alkaloid of cinchona from Cinchona tree [36] and artemisinin, a sesquiterpene lactone endoperoxides derived from Qing hao (*Artemisia annua*) [36,37]. The observed low EC50 value of methanol extract as compared to the ether extract could possibly be due to the presence of high quantities of alkaloids, steroids and triterpenoids such as sesquiterpene that were extracted by the methanol solvent in large quantities [35,36,38]. Previous studies on the *in vitro* antiplasmodial activities of the root-bark extracts of *Z. chalybeum* have shown strong activities of the herb against *P. falciparum* organisms [34,39]. However, the continued use of the root-bark of the herb by the local communities and traditional herbalist in treatment of malaria does not conserve the plant since this method destroys the plant and hence leading to eventual extinction. As a result of this problem, the study utilized the leaves of the specie that also showed to have antiplasmodial activity though the EC50 values were slightly higher as compared to the previous studies that utilized the root-bark of the plant [37-39]. And the difference could possibly be due to the accumulation of the active secondary metabolites in the root-bark as compared to the leaves. However, the high EC50 value of chloroquine diphosphate as compared to the methanol and ether extracts of *Z. chalybeum* may be due to the resistance wild of *P. falciparum* protozoal organisms to chloroquine diphosphate that would require a high concentration of the drug to cause the suppression of 50% schizonts development, poor quality, expired drug or it had undergone degradation thus reducing its activity. Though the EC50 value of chloroquine diphosphate obtained from the study correlates with the values obtained from previous studies [37,38,40], the culture medium used as negative control did not suppress the *P. falciparum* schizonts development and this was due to lack of active compound with antiplasmodial activity in the culture medium. The results therefore showed that the ether and methanol leaf extracts of *Z. chalybeum* herb contains various secondary metabolites with antiplasmodial activity and hence the reason for its use as a decoction and concoction [32,35] by the local communities and traditional herbalist in the treatment of malaria in Uganda.

It was therefore concluded that the ether and methanol leaf extracts of *Z. chalybeum* contains secondary metabolite compounds with antiplasmodial activity and this could be the reason why they are used by traditional herbalists and local communities in Uganda in the management and treatment of malaria. The leaves of the herb also contain active secondary metabolites against *P. falciparum* and hence can be utilized as an alternative in the treatment of malaria as opposed to the root-

bark whose method of harvesting does not favor the sustainability and survival of the plant predisposing it to extinction.

Musila et al. [39] worked on the *in vivo* anti-malarial activity, toxicity and phytochemical screening of selected anti-malarial plants. Previous studies have shown that water and methanol extracts of the stem bark of *Z. chalybeum* have significant *in vitro* antimalararial activity against chloroquine sensitive and chloroquine resistance strains of *P. falciparum* which is in line with the results on *Z. chalybeum* obtained in the current study. Methanolic extracts of the root bark of *Z. chalybeum* have been reported to have significant anti-malarial activity on *P. falciparum* [34]. This is also in agreement with the observations in the current study. In other studies, aqueous extract of a related species *Zanthoxylum usambarense* also exhibited significant antiplasmodial activity against *P. falciparum* [41].

T. asiatica

Orwa et al. [42] investigated aqueous, ethyl acetate, hexane and methanol extracts obtained from *T. asiatica* root bark, fruits and leaves. *In vitro* antiplasmodial activity was done using chloroquine-sensitive (D6) and chloroquine-resistant (W2) *P. falciparum* strains and the concentration causing 50% inhibition of radioisotope incorporation (IC(50)) was determined. *In vivo* assay was done by administering mice infected with *Plasmodium berghei*. Four consecutive daily doses of the extracts through oral route following Peters 4-Day suppressive test. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in untreated control with those of treated mice. Quinine hydrochloride was used as positive control while double distilled water or 20% Tween-80 was used as a negative control. *In vivo* acute toxicity was determined in mice using standard procedures. *In vitro* cytotoxicity assay was carried out using actively dividing sub-confluent Vero cells.

Inhibitory concentrations of ethyl acetate extract of *T. asiatica* fruits showed high activity against chloroquine resistant (W2) strains of *P. falciparum* (IC(50)=1.87 µg/ml), followed by root bark aqueous extract (IC(50)=2.43 µg/ml). Tested *in vivo* against *P. berghei*, the fruit ethyl acetate extract (500 mg/kg) and root bark aqueous extract (250 mg/kg) reduced malaria parasitaemia by 81.34% and 56.8% respectively. Higher doses were found to be less effective *in vivo*. Acute toxicity and cytotoxictiy of the tested extracts, with the exception of hexane extract from the roots, showed LD (50)>1000 mg/kg and CC (50)>100 µg/ml respectively.

The results obtained contribute to the validation of traditional use of *T. asiatica* and provides *in vivo* and safety data of the plant extracts tested for the first time. Ethyl acetate extract of the fruits was active against chloroquine resistant *P. falciparum* as well as against *P. berghei*. These findings confirm the suitability of *T. asiatica* as a good candidate for further tests to obtain a prototype for anti-malarial medicine.

Molmoori et al. [43] did a review on *T. asiatica*. In their paper, it was stated that the root bark of the plant was fractionated using ethyl acetate, dichloromethane, methanol, water and was studied for *in vitro* anti-malarial activity against chloroquine-susceptible strains (K67, K39, M24, UPA, SL/D6, HB3) and chloroquine-resistant strains (ItD12, FCR3, FCB, V1/S) of *P. falciparum* and compared with nitidine. It was found that the methanolic extract showed highest activity and nitidine was two orders of magnitude more potent than the crude extract [44]. The antiplasmodial activity of the crude extract was attributed to 5,7-dimethoxy-8(3'-hydroxy-3'-methyl-1'- butene)-coumarin which was isolated from bioactivity-guided fractionation of the ethyl acetate extract of the plant [21,45-49].

DISCUSSION

From previous works which has mostly been based on drug (chloroquine) resistant *Plasmodium*, it has been shown that these three plant *C. anisata*, *Z. chalybeum* and *T. asiatica* have anti-malarial activities. It has also shown that *Z. chalybeum* might be toxic to the body if the dosage is not regulated. These plant species possess Alkaloids which inhibit the growth and functioning of the *Plasmodium* in the system. Although, more research is needed to investigate the effectiveness and toxicity of these plants, it can be said that these three species can be used in the cure and possible eradication of malaria in affected countries.

COMPETING INTEREST

Authors declare no competing interest.

REFERENCES

1. Caraballo H and King K. Emergency department management of mosquito-borne illness: Malaria, dengue, and West Nile virus. Emerg Med Pract. 2014;16:1-23.
2. Nadjm B and Behrens RH. Malaria: An update for physicians. Infect Dis Clin North Am. 2012;26:243-59.
3. Mark CW, et al. Phylogenetic relationships of Rutaceae: a cladistic analysis of the subfamilies using evidence from RBC and ATP sequence variation. Am J Bot. 1999;86:1191-1199.
4. Letouzey R and du Gabon F. Rutaceae, Zygophyllaceae, Balanitaceae. National Museum of Natural History. Paris, France. 1963;6.
5. Hamza OJM, et al. Plants used to treat epilepsy by Tanazanian traditional healers. J Ethnopharmacol. 2005;97:327-336.

6. Umar MB, et al. Antiplasmodial Efficacy of Methanolic Root and Leaf Extracts of Morinda lucida. J Nat Sci Res. 2013;3:112-122.
7. Maneerat W, et al. Bioactive carbazole alkaloids from Clausena wallichii roots. J Nat Prod 2012;75:741-746.
8. Joshi BS, et al. Structure of heptaphylline, a carbazole alkaloid from Clausena heptaphylla Wt. & Arn. Tetrahedron Lett. 1967;8:4019-4022.
9. Lakshmi V, et al. Monoterpenoid furanocoumarin lactones from Clausena anisata. Phytochemistry 1984;23:2629-2631.
10. Ngadjui BT, et al. Prenylated coumarins from the leaves of Clausena anisata. J Nat Prod. 1989;52:243-247.
11. Ngadjui BT, et al. Limonoids from Clausena anisata. J Nat Prod. 1989;52:832-836.
12. Orwa C, et al. Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya. 2009.
13. Maundu PM, et al. Traditional food plants of Kenya. Kenya Resource Centre for Indigenous Knowledge. National Museums of Kenya. 1999.
14. Mingochi DS and Luchen SWS. Traditional vegetables in Zambia: genetic resources, cultivation and uses. In: Traditional African Vegetables, Proceedings of the IPGRI International Workshop on Genetic Resources of Traditional Vegetables in Africa: Conservation and Use, Guarino L (ed), Nairobi, Kenya. 1995.
15. Engeu OP, et al. Repeat-dose effects of Zanthoxylum chalybeum root bark extract: a traditional medicinal plant used for various diseases in Uganda. Afr J Pharma Pharmacol. 2008;2:101-105.
16. Nibret E, et al. Screening of some Tanzanian medicinal plants for their trypanocidal and cytotoxic activities. Phytother Res. 2010;24:945-947.
17. Sharma BD, et al. Flora of India. 1993.
18. Njoroge GN and Bussmann RW. Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). J Ethnobiol Ethnomed. 2006;2.
19. Nabwami J, et al. Characterization of the natural habitat of Toddalia asiatica in the Lake Victoria basin: soil characteristics and seedling establishment. Afr Crop Sci Conf Proc. 2007;8.
20. Bussmann RW, et al. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. J Ethnobiol Ethnomed. 2006;2:22.
21. Oketch-Rabah HA, et al. A new antiplasmodial coumarin from Toddalia asiatica roots. Fitoterapia. 2000;71:636-640.
22. Schlitzer M. Review; Antimalarial Drugs-what is in use and what is in the pipeline. Arch Pharmacol Chem. 2008;341:149-163.
23. Okwu DE. Phytochemicals, Vitamins and Mineral Contents of Two Nigerian Medicinal Plants. Int J Mol Med Adv Sci. 2004;1:375-381.
24. Pascual ME, et al. Lippia: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol. 2001;76:201-214.
25. Noedi H, et al. Malaria drug sensitivity: new assays, new perspectives. Trends Parasitol. 2003;19:175-181.
26. Jude EO, et al. Antiplasmodial and analgesic activities of Clausena anisata. Asian Pac J Trop Med. 2012;214-219.
27. Johnson DJ, et al. Evidence for a Central Role for PfCRT in Conferring Plasmodium falciparum Resistance to Diverse Antimalarial Agents. Mol Cell. 2004;15:867-877.
28. Beatrice N, et al. In vivo antimalarial and acute toxicity properties of hexane and chloroform extracts from Clausena anisata (Wild.) Benth. Afr J Pharmacol Ther. 2012;1:24-29.
29. Christensen SB and Kharazmi A. Antimalarial natural products. Isolation, characterization and biological properties. In: Tringali C (ed) Bioactive compounds from natural sources: Isolation, characterization and biological properties. Taylor & Francis, London, UK. 2001:379-432.
30. Bentley GA, et al. Studies on the antinociceptive action of agonist drugs and their interaction with opoid mechanisms. British J Pharma. 1983;79:125-134.
31. Houghton JP and Raman A. Laboratory handbook for fractionation of natural extracts. 1st ed. Chapman and Hall, London, UK. 1998:55-65.
32. Godfrey BS, et al. Activity of Leaf Extracts of Zanthoxylum chalybeum Engl. Br J Pharm Res. 2014;4:705-713.
33. Emerole G, et al. Structure-activity relationship in the toxicity of some naturally occuring coumarins-chalepin, imperatorin and oxypeucedanine. J Toxicol. 1981;20:71-80.
34. Uwaifo AO. The mutagenicities of seven coumarin derivatives and a furan derivative (nimbolide) isolated from three medicinal plants. J Toxicol Environ Health 1984;13:521-530.
35. Ito C, et al. Gamma-lactone carbazoles from Clausena anisata. J Nat Prod 2009;72:1202-1204.
36. Nguta JM, et al. Antimalarial herbal remedies of Msambweni, Kenya. J Ethnopharmacol. 2010;128:424-432.

37. Patiño LOJ, et al. Zanthoxylum Genus as Potential Source of Bioactive Compounds in Phytomedicine. In: Rasooli I (ed). InTech, Rijeka, Croatia. 2012.

38. Muganga R, et al. Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. J Ethnopharmacol. 2010;128:52-57.

39. Musila MF, et al. In vivo antimalarial activity, toxicity and phytochemical screening of selected antimalarial plants. J Ethnopharmacol. 2013;146:557-561.

40. Brunton LL, et al. Chemotherapy of Malaria: Artemisinin and Derivatives; Quinolines and Related Compounds. Goodman & Gilman's. The Pharmacological Basis of Therapeutics, 12th ed. McGraw Hill Education, New York, USA. 2013.

41. Ferreira JFS, et al. Artemisinin concentration and antioxidant capacity of Artemisia annua distillation byproduct. Ind Crops Prod. 2012;41:294-298.

42. Orwa JA, et al. Antimalarial and safety evaluation of extracts from Toddalia asiatica (L) Lam. (Rutaceae). J Ethnopharmacol. 2013;30:587-90.

43. Molmoori R, et al. Toddalia asiatica (Linn.) Lam. A Comprehensive Review. Phcog Rev. 2008;2:386-397.

44. Dahnum D, et al. Isolation of Artemisinin as Antimalarial Drugs from Artemisia annua L. Cultivated in Indonesia. Int J Basic Appl Sci. 2012;12:90-95.

45. Rukunga GM, et al. Antiplasmodial activity of the extracts of some Kenyan medicinal plants. J Ethnopharmacol. 2009;121:282-285.

46. Appiah-Opong R, et al. Antiplasmodial activity of extracts of Tridax procumbens and Phyllanthus amarus in vitro Plasmodium falciparum culture systems. Ghana Med J. 2011;45:143-150.

47. Kirira PG, et al. Anti- plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi districts of Kenya. J Ethnopharmacol. 2006;106,403-407.

48. Gakunju DMN, et al. Potent Antimalarial Activity of the Alkaloid Nitidine, Isolated from a Kenyan Herbal Remedy. Antimicrob Agents Chemother 1995;39:2606-2609.

49. Nyangulu JM, et al. Antimalarial benzo[c]phenanthridines. Bioorg Med Chem Lett. 2005;15:2007-2010.