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

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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 [6]. 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].
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 antimalarial 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
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 PGF2 and PGE2 [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 (10.62-16.59) µg/ml at 95% CI; methanol extract had EC50 value of 8.10 (5.89-11.12) µg/ml and chloroquine diphosphate had EC50 value of 25.33 (17.07-37.60) µg/ml. The observed low effective concentration (EC50) of methanol extract as compared to the ether extract and chloroquine diphosphate as control, could have been due to the presence of high quantities of alkaloids, steroids and triterpenoids such as 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 species 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 antimalarial 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 usambarensense 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 cytotoxicity 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.

Molmooiri 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


