Antimalarial activity of *Artemisia nilagirica* against *Plasmodium falciparum*

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Abstract Malaria is one of the most prevalent vector borne infectious disease and a serious global health problem in the world. Treatment for malaria is commonly inadequate due to the lack of quality assured limited number of effective drugs, underline how important it is to discover new antimalarial plants from number of natural sources. In the present study, the efficacy of antimalarial activity was studied by taking six various (n-hexane, chloroform, petroleum ether, ethanol, methanol and aqueous) organic leaf extracts of *Artemisia nilagirica* (Clarke) Pamp. against malarial parasite *Plasmodium falciparum*. Promising antiplasmodial activity was found in all tested extracts; however, maximum 50% inhibitory concentration (IC₅₀) values were noticed after 32 h of incubation, which is 5.76 ± 0.82 , 7.09 ± 1.09 , 9.88 ± 1.13 , 10.24 ± 1.52 , 11.37 ± 1.77 and 50.15 ± 6.16 µg/ml in methanol, chloroform, n-hexane, petroleum ether, ethanol and aqueous extracts, respectively. In conclusion, *A. nilagirica* leaf extract possesses antiplasmodial activity which may be used as a potent plant-based antimalarial drug in the future by investigating the hidden phytochemical/(s).

Keywords Antimalarial activity · *Artemisia nilagirica* · *Plasmodium falciparum* · Inhibitory concentration (IC₅₀)

Introduction

The death proportion is now associated with malaria and about 600 million people are at risk of infection and are killed approximately 1.2–2.7 million per year (Adetutu et al. 2016). In particular, young children (below 5 years), pregnant women and immunodeficient persons are at greatest risk of severe or fatal illness (Kodippili et al. 2011). The disease is caused by infection with five plasmodian protozoan species such as *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. However, the majority of the mortality from malaria is due to the infection with *Plasmodium falciparum* and may be because of resistance to some classes of antimalarial drugs acted upon it (Hafid et al. 2016). The highest number of malaria and malaria-related death are recorded from the state Odisha, located in the eastern part of India is also due to infection of *Plasmodium falciparum* (Kumar et al. 2007).

Natural products are being used as the traditional medicine, which contain a larger variety of chemical compounds and have been screened for antiplasmodial activities as potential sources of new antimalarial drugs (Ginsburg and Deharo 2011). Also, plant derived products have been increasingly used for mosquito control because of their efficacy and as well as non-toxic effects on infected organisms (Oliveira et al. 2009). As per WHO recommendation, artemisinin-based combination therapy (ACTs) is a first-line of treatment for uncomplicated *P. falciparum* malaria in various developing countries in a routine fashion. Trials were still under testing for the efficacy of

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current ACTs and some other combinations (Kodippili et al. 2011). However due to dispersion as well as resistance properties, now malaria is an important global public health problem, which encourages the search for a new antimalarial drug.

Artemisia nilagirica is an important medicinal plant, which belongs to the family Asteraceae and is popularly known as Indian Wormwood (Suresh et al. 2011). The plant is popularly known in India by different vernacular names like Davana (Hindi), Dayona (Odia), Nagadana (Bengali), Leibakngou (Manipuri), Gondhomaro (Marathi), Masipatri (Telugu) etc. (Anonymous 2012). It is a green shrub found throughout the hilly areas of India, Afghanistan and Japan. In India, it is widely grown in Himachal Pradesh, Uttarakhand, Rajasthan, Tamil Nadu, Kerala, West Bengal and Sikkim (Joshi et al. 2016). It is widely used as anti-inflammatory, skin disorders, antiasthmatic, antimicrobial, antifungal, anticancer, antiulcer, antioxidant, mosquito larvicidal and repellent activities (Suresh et al. 2011; Panneerselvam et al. 2012; Rani et al. 2012; Sati et al. 2013; Stappen et al. 2014) however, least evidences are found regarding its antimalarial activity (Valecha et al. 1994; Shukla et al. 2015). Therefore, the present study is designed to evaluate the antimalarial activity of leaf extract of *A. nilagirica* against *P. falciparum*.

Materials and methods

Collection of plant materials

The plant materials were collected from Nilgiri hills, Tamil Nadu, India. The taxonomic identification of collected samples was made by the exports of Post Graduate Department of Botany, Utkal University. The plants were maintained in the botanical garden of Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha, India. The healthy selected leaves were taken for the experiment.

Preparation of plant extracts

The collected samples were washed in running tap water and air dried for 10–15 days in the shade at an environmental temperature (27–37 °C). The leaves (250 g) were powdered mechanically using a commercial electrical stainless steel blender and extracted with n-hexane, chloroform, petroleum ether, ethanol, methanol and aqueous (distilled water) in a Soxhlet apparatus (boiling point range 60–80 °C) for 72 h. Extracts were concentrated (0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/ml) on a rotary evaporator under a reduced pressure of 22–26 mmHg at 45 °C

and the residues obtained were weighed and stored at −4 °C.

Collection and processing of serum

AB positive blood obtained (usually collected as packs) from the blood bank of Shri Ramachandra Bhanj Medical College, Cuttack, Odisha. Blood was removed aseptically and centrifuged at 2500 rpm to separate serum from red blood cells. Serum was carefully decanted off and stored frozen in large sterile plastic 1 litre bottles. Serum from a minimum of 5 (preferably 7) donors is pooled before being liquated in 40 or 50 ml aliquots at −20 °C. Serum was then inactivated by keeping at 56 °C water bath for 30 min. After inactivation, the serum was stored in a deep freezer at −20 °C for further use.

In vitro cultivation of *P. falciparum*

P. falciparum (FCR-3 strain) were used for in vitro blood stage culture to test the antiparasitic efficacy of different plant extracts. The continuous culture was maintained at the Regional Medical Research Centre (RMRC), Bhubaneswar, Odisha, India. *P. falciparum* culture was maintained according to the method described by Trager and Jensen (1976) with minor modifications that in fresh 10% AB positive human serum suspended at 5% haematocrit in RPMI-1640 (Sigma) containing 0.2% sodium bicarbonate, 25 mM HEPES buffer, glucose and 40 µg/L gentamicin sulphate. The culture was kept in CO₂ incubator at 37 °C. Regularly, the infected erythrocytes were transferred into freshly prepared complete medium to propagate the culture.

Antiparasitic activity evaluation

After incubation for various hours (0, 8, 16, 24, 32, 40, 48, 56, 64, 72 h), contents of the wells were harvested and stained. The growth stages of parasites were monitored by making a thin blood smear, fixed with methanol and stained with Giemsa 10% for 30 min. The parasitemia was examined under the microscope using magnification 1000X by adding immersion oil. Percentage parasitemia was then calculated by dividing the number of infected-RBC by the total number of RBCs indexed and multiplied by 100. But for the percentage growth inhibition of the parasites was calculated by dividing the mean parasitemia of control minus the mean parasitemia of treated group by the mean parasitemia of negative control group and multiplied by 100 (Kalra et al. 2006). The percentage of the growth stages inhibition of the parasites at each concentration was determined by the mean of at least three IC₅₀ parasites

Table 1 The 50% inhibitory concentration (IC₅₀) values of different solvents extraction of *A. nilagirica* on growth of *P. falciparum*

IC ₅₀ (μg/ml)						
Observation (h)	Different solvents used for leaf extracts					
	Methanol	Chloroform	n-Hexane	Petroleum ether	Ethanol	Aqueous
8	21.93 ± 3.16	22.74 ± 73.49	24.51 ± 3.14	25.92 ± 3.04	27.09 ± 3.46	62.21 ± 7.49
16	34.19 ± 3.88	37.34 ± 3.75	39.34 ± 4.26	41.2 ± 4.50	43.81 ± 5.03	75.92 ± 10.59
24	10.24 ± 1.43	13.44 ± 1.54	17.86 ± 1.77	18.43 ± 2.08	20.54 ± 2.59	55.25 ± 7.03
32	5.76 ± 0.82	7.09 ± 1.09	9.88 ± 1.13	10.24 ± 1.52	11.37 ± 1.77	50.15 ± 6.16
40	16.35 ± 2.01	16.04 ± 1.65	20.25 ± 3.01	21.17 ± 3.34	21.78 ± 2.91	58.46 ± 5.21
48	26.66 ± 3.74	31.18 ± 3.83	35.12 ± 4.05	37.56 ± 4.11	39.71 ± 5.76	73.83 ± 9.11
56	96.18 ± 13.22	106.37 ± 17.48	112.26 ± 15.33	104.09 ± 20.10	110.82 ± 18.16	143.59 ± 27.51
64	93.96 ± 11.06	97.84 ± 12.26	98.48 ± 12.67	99.31 ± 12.89	100.73 ± 11.12	128.04 ± 23.09
72	23.98 ± 2.66	24.62 ± 3.22	25.16 ± 2.82	27.34 ± 3.34	28.55 ± 3.46	65.06 ± 8.01

The data represents mean ± SE of replicates (n = 6)

viability. The 50% of growth stages inhibition was calculated using Probit analysis.

Statistical analysis

All results are the mean of three independent experimental replicates (n = 6) and data is reported as mean ± standard error.

Results

The various organic (n-hexane, chloroform, petroleum ether, ethanol, methanol and aqueous) leaf extracts of *A. nilagirica* were assayed for antimalarial activity against *P. falciparum* FCR-3 strain. For that, percentage parasitemia, percentage growth inhibition and IC₅₀ values were analyzed after a different time interval (0, 8, 16, 24, 32, 40, 48, 56, 64, 72 h) of incubation. It was observed that, all tested leaf extracts showed a significant IC₅₀ value. However, the maximum inhibition (5.76 ± 0.82 μg/ml) was observed in methanol extract of 32 h of incubation, then followed by chloroform (7.09 ± 1.09 μg/ml), n-hexane (9.88 ± 1.13 μg/ml), petroleum ether (10.24 ± 1.52 μg/ml), ethanol (11.37 ± 1.77 μg/ml), aqueous (50.15 ± 6.16 μg/ml) (Table 1).

As the methanolic extract showed maximum activity, the percentage of parasitemia and growth inhibition data on the same extract was presented in this study. When studied the percentage of parasitemia and percentage of growth inhibition against *A. nilagirica*, a remarkable result was detected and was not only dependent on types of the solvent but also the concentration of leaf extracts (0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 μg/ml) and the duration of incubation (Figs. 1, 3). The best inhibited parasitemia percentage was occurred in 200 μg/ml concentration of methanolic extract of 8 and 32 h of

Fig. 1 The percentage of parasitemia tested for *P. falciparum* in different concentrations of methanolic leaf extracts of *A. nilagirica* after cultivation from 8 to 72 h

incubation which was 2.32 and 3.02%, respectively (Fig. 1). The variation of numerous parasitized RBC and few normal RBC were also visualized microscopically in different stages of the experiment and were presented in Fig. 2. Similar findings were also observed in percentage of growth inhibition, that 200 μg/ml concentration of methanolic extract yielded maximum inhibition activity followed by 100, 50, 25, 12.5, 6.23, 3.125 μg/ml of concentration however, maximum growth inhibition was found after 32 h of incubation in all tried concentrations (Fig. 3).

Discussion

Malaria is a serious global health problem across the world, which imposes a great socio-economic burden on humanity. It is a major vector-borne disease, alarming drug resistance and has limited numbers of effective drugs. The situation is now drastic as neither a consistent drug nor an approved vaccine is available for malaria (Kumar et al.

Fig. 2 Level of parasitemia showing microscopically from methanolic extract of *A. nilagirica* on growth of *P. falciparum*. **a** Control (without plant extract); **b** After 8 h of incubation; **c** After 16 h of incubation; **d** After 24 h of incubation; **e** After 32 h of incubation;

f After 40 h of incubation; **g** After 48 h of incubation; **h** After 56 h of incubation; **i** After 64 h of incubation; **j** After 72 h of incubation

2007; Hill 2011). Due to the emergence and spread of malaria parasite with resistance to antimalarial drugs, discovery and development of alternative options are urgently

needed. Therefore, the present study was aimed to determine the antimalarial capability of extracts of *A. nilagirica* against *P. falciparum* under in vitro condition.

Fig. 3 The percentage of growth inhibition of *P. falciparum* in different concentrations methanolic leaf extracts of *A. nilagirica* after cultivation from 8 to 72 h

Different strategies are available for the initial screening of new antimalarial drugs from plant species. However, in vitro anti-plasmodial inhibitory activity and identification of particular type of crude extract exhibiting IC_{50} values less than $10 \mu\text{g/ml}$ are key important steps towards searching of new anti-malarial drugs (Lima et al. 2015). In vitro antiplasmodial IC_{50} values can be classified as active ($\leq 10 \mu\text{g/ml}$), moderately active (10 to $\leq 25 \mu\text{g/ml}$) and inactive ($>25 \mu\text{g/ml}$) [Kumari et al. 2016; Lima et al. 2015]. Various extracts such as n-hexane, chloroform, petroleum ether, ethanol, methanol and aqueous were tried to achieve the effectiveness of activity and based on this classification active results were obtained from methanol, chloroform and n-hexane. However, methanol extracts were very effective when compared to others (Table 1) and this would be credited to the fact that methanol extracts may contain the bioactive compounds and chemical constituents which are responsible for strong antiplasmodial activity (Jeruto et al. 2015; Kumari et al. 2016). It was reported that the major ingredients known to occur in most medicinal plants are the phenols, alkaloids, terpenoids, coumarins, polysaccharides and flavonoids, whose presence is responsible for antimalarial activity in plants (Oliveira et al. 2009; Kodippili et al. 2011; Adetutu et al. 2016). Our findings are in agreement with those of others on screening of different medicinal plant species such as *Guiera senegalensis*, *Phyllanthus emblica*, *Syzygium aromaticum*, *Neurolaena lobata*, *Nectandra membranacea*, *Hampea appendiculata*, *Psidium guajava*, *Bocconia frutescens*, *Siparuna thecaphora*, *Witheringia solanacea*, *Strachium sparganophorum*, *Tithonia diversifolia*, *Pycnanthus angolensis* and *Morinda lucida* for antimalarial activity; however, the results differ on the plant parts and solvent system used (Azas et al. 2002; Madureira et al. 2002; Bagavan et al. 2011; Chinchilla et al. 2012).

Plants have over the years, proved to be a good source of chemotherapeutic agents and according to WHO, 80% of world's population depends on plants for their primary

health care (Oliveira et al. 2009). In order to deliver better drugs against malaria, medicinal plants also have been the best sources since 1820 and considered as the first effective treatment against plasmodium species in the form of quinine which was isolated from the bark of *Cinchona* species. In 1972, another consistent drug Artemisinin was extracted from *Artemisia annua* and its semi synthetic derivatives are used till today (Saxena et al. 2003; Mojab 2012). One more species, *Dichroea febrifuga* is used as an antimalarial agent and its active compound “febrifugine” is currently under clinical trial (Mojab 2012). The latest antimalarial drug is atovaquone, which is extracted from *Tabebuia impetiginosa*. But now, it is uncommon due to its high cost (Tar-kang et al. 2016).

Conclusion

In conclusion, our study shows that the methanol extract of leaf of *A. nilagirica* exhibits good in vitro antiplasmodial activity against *P. falciparum* FCR-3 strain. The results of this study provide a basic knowledge for further work on searching of a potent antimalarial agent. Further, isolation and characterization of bioactive constituents from leaf extract may provide potential molecule responsible for therapy of malaria. Additionally in vivo work is essential to understand the mechanisms of action of the active plant extracts.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval statement The experimental aims and protocol were explained to the director and head of molecular epidemiology of RMRC, Bhubaneswar. Informed consent was obtained from all of the individual patients that were included in the study.

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