Phytochemical screening of ethanolic leaf extract of eichhornia crassipes for antimalarial activity

Samidoss Christina Mary*[1], S Mary Joy[2] and K. Murugan[1]

[1]*Department of Zoology, School of Life sciences, Bharathiar University, Coimbatore- 46, Tamil Nadu, India.*
[2] *Department of Biochemistry, PSG College of Arts and Science, Coimbatore- Tamil Nadu, India.*

Abstract

Malaria is a parasitic infection caused by a parasite that spends part of its life in people and the rest in mosquitos. Malaria continues to be one of the world's worst killers, threatening the lives of more than a third of the world's population. Treatments with organophosphates and insect growth regulators are the main control tools against Anopheles larvae, but they have negative effects on human health and the environment. Green control tools are a priority in this circumstance and are required for mosquito control. In this present study, Positive and negative controls were orally provided in mice for 24 hours before several tests were conducted out in the current investigation to evaluate the Treatment of Ethanolic extracts of *Eichhornia crassipes*. Mice were used in the Acute Toxicity Tests, the Early Malaria Infection Test, and the Established Infection Method Test. Asthenia, piloerection, ataxia, anorexia, urination, diarrhoea, lethargy, and coma were among the behavioural signs of toxicity observed in the mice. As a result, *Eichhornia crassipes* extract appears to have significant malarial activity. As a result, *Eichhornia crassipes* could be used as a natural antiplasmodial agent for the fight against Malaria.

Keywords: Malaria, *Eichhornia crassipes*, toxicity, mice, antiplasmodial.

***Corresponding author Email:** 25chrisento@gmail.com

1. Introduction

Mosquitoes (Diptera: Culicidae) pose a major threat to millions of people worldwide, as they vector important parasites and pathogens, including malaria, dengue, and filariasis (Mehlhorn *et al.*, 2012; Benelli 2015a). Malaria mortality rates have fallen by 47 % globally since 2000 and by 54 % in the African region. Most deaths occur among children living in Africa, where achild dies every minute from malaria. Malaria mortality rates among children in Africa have been reduced by an estimated 58 % since 2000. Malaria is caused by Plasmodium parasites, vectored to people through the bites of infected Anopheles mosquitoes, which bite mainly between dusk and dawn (WHO 2014).

Phytochemicals have a major role in mosquito control programs. The bioactive plant ingredient(s) can be obtained from the whole plant or from a specific part by extraction with different types of polar and nonpolar solvents, such as petroleum ether, benzene, chloroform, methanol, absolute alcohol, acetone, etc. *Eichhornia crassipes* is an invasive plant that is native of the Amazon basin (Barret and Forno 1982) and whose capacity for growth and propagation causes major conservation problems with considerable socioeconomic repercussions and it is a free-floating plant found growing in almost all the aquatic environment of Asia Pacific region. *Eichhornia crassipes* (Water hyacinth) of a Pontederiaceae family, a native of South America, is one of the free floating macrophytes found in the aquatic environment such a ditches, ponds, and lakes, is mostly studied for the purposes of phytoremediation (Melignani *et al.,* 2015) because of its easily cultivable under heavy metal stress and could produce high biomass in aquatic environment without showing much toxic symptoms (Malar *et al.,* 2015). It is also responsible for drastic changes in the plant and animal communities of freshwater environments and acts as an agent for the spread of serious diseases in tropical countries. The impact of *E. crassipes* on the physico-chemical characteristics of the water in general are declines in temperature, pH, biological oxygen demand (organic load), and nutrient levels (Rai and Datta Mushi 1979). Sometimes there is a complete decline of dissolved oxygen, leading to the deaths of a great number of fishes. The invasive and infesting nature of this plant disturbed the whole environment wherever if present and has become one of the most problematic environmental concern. The current work discussed the cost-effective and eco-friendly way of utilizing this invasive and infesting plant in a way to incur the daily needs and also help in controlling the negative outcome (Prabhat Kumar Rai and Mayanglambam Muni Singh, 2016).

Molecular biology, genetic engineering and computational chemistry have created considerable potential within the pharmaceutical industry without the need to explore nature's chemical diversity. In the synthetic drug development of compounds, both a search is made through the inventory of substances earlier synthesized to find relatives to the theoretical molecule, or the theoretical molecules and analogues are synthesized. People entering into regions where arbovirus risk exists may protect themselves by use of chemical- or plant-derived repellents (Mehlhorn *et al*. 2005; Mehlhorn 2011; Amer and Mehlhorn 2006a, b), while people living in endemic regions have to protect themselves by several strategies at the same time, since the daily infection rates of mosquitoes may be extremely high (Amer and Mehlhorn 2006c). In recent years, important efforts have been conducted to propose plant-borne compounds as valuable alternatives to synthetic mosquitocides, due to their effectiveness, reduced toxicity towards vertebrates, and high biodegradability (e.g., Panneerselvam *et al.* 2013; Murugan *et al*. 2015a; see Benelli *et al.* 2015a, b, and Pavela 2015 for reviews). Moreover, an essential oil extracted from *M. dianthera* aerial parts was reported to demonstrate promising anticancer effect against all cancer cell lines (IC_{50} values ranged from 83.6 to 91.2 µg/ mL) and considerable antibacterial, antifungal, and antioxidant properties (Mothana *et al.,* 2019). Furthermore, methanolic leaves extract of *M. dianthera* showed significant *in vitro* α-amylase and α-glucosidase inhibitory activities as well as *in vivo* antidiabetic effect in alloxan-induced diabetic rats (Mussie *et al.,* 2015; Sium *et al.,* 2017).

1.1 Distribution

Eichhornia crassipes is an invasive plant that is native of the Amazon basin (Barret and Forno 1982), has distributed all over the world and has invaded Asia, Africa, Australia, Europe and North America (Téllez

et al., 2008; Shanab *et al.*, 2010). In South America, its presence was reported in 1902 from Brazil, from Argentina in 1942, from Paraguay, Uruguay, Bolivia, Equador and Columbia in 1959, from Venezuela in 1976, and from Chile in 1979 (Téllez *et al.*, 2008). In India, the plant was first introduced from Brazil as an ornamental plant in the year 1896 (Rao, 1988).

1.2 Importance

It has been mostly studied for its tendency to bio-accumulate and biomagnify the heavy metal contaminants present in water bodies (Tiwari *et al.*, 2007). The potential application of this plant in the removal of heavy metals from water was discovered in the early 1980s (Prabhat Kumar Rai *et al.*, 2015). It accumulates metals and as the recycling process is run by photosynthetic activity and biomass growth, sustainable process and cost efficient (Garbisu *et al.*, 2002; Bertrand and Poirier, 2005). Due to its exotic invasive nature and rapid decomposition in comparison to other plants, it has been reported that the growth of water hyacinth poses problem in functioning in the aquatic ecosystem e.g. constructed wetlands (Khan *et al.*, 2000; Rai, 2011, 2012).

1.3 Phytochemicals Composition

Eichhornia crassipes being a fast-growing plant is used for rapid removal of various kinds of pollution in water resulting in positive outcomes. The plant was evaluated for its possible potential of heavy metal accumulations which results in the discovery of high cellulose content and its functional groups including amino (-NH2), carboxyl (-COO-), hydroxyl (-OH-), sulfahydryl (-SH) showing high tolerance and affinity towards heavy metals adsorption (Patel, 2012). *Eichhornia crassipes* contains many phytochemicals such as amino acids including glutamic acid theonine, leucine, lysine, methionine, tryptophan, tyrosine, and valine; flavonoids including apigenin, azaeleatin, chrysoeriol, gossypetin, kaempferol, luteolin, oientin and tricin (Nyananyo *et al.*, 2007). The dry mass of the plants is consisted of 5.2% nitrogen, 0.22% of phosphorous, 2.3% of potassium, 0.36% of calcium, 280 ppm of Iron, 45 ppm of Zn, 2 ppm of Cu and 332 of Mn (Koutika and Rainey, 2014).

Thus, the search has been directed extensively to the plant kingdom as many plant chemicals have larvicidal, pupicidal, and adulticidal activities, most being repellants, ovipositional deterrents, antifeedants and antimalarial against both agricultural pests and medically important insect species. It is in this context the present bioassay was for antimalarial activity were carried out in the Entomology Research Lab of Zoology Department at Bharathiar University, Coimbatore. In the present study; we describe the effect of *Eichhornia crassipes* leaf extracts against the malarial vector, *A. stephensi*. The aim of this study was to investigate the antimalarial activities of different solvent extracts of *Eichhornia crassipes* from Coimbatore District, Tamil Nadu, India.

2. Materials and Methods

2.1 Plant extracts

The plant *Eichhornia crassipes* was collected in its natural habitat in Singanallur pond, Coimbatore, Tamil Nadu, India and the herb was air-dried and ground to provide a fine powder. Extracts were then prepared by soxhlation of the powder with methanol solvent. Two hundred grams of the powder was soxhalated with 1,000 ml of methanol for 24 hours. Upon evaporation under reduced pressure, methanolic extracts were obtained.

2.2 Preliminary Phytochemical analysis

Phytochemical screening of the *Eichhornia crassipes* extracts were carried out using standard procedures to test the presence of alkaloids, saponin glycosides, cardenolides, flavonoids, tannins, polyphenolic compounds, anthraquinones (Lata N and Venapani Dubey, 2010)

2.3 Parasites and Inoculum

P. falciparum were used to assess the *in-vivo* intrinsic antimalarial activity. The test protocol was based on the 4-day suppressive test described by (Peters *et al.*, 1975). Parasite strain was maintained by serial passage of blood from mouse to mouse. A standard inoculum of 1×107 of parasitized erythrocytes from a donor mouse in volumes of 0.1ml was used to infect the experimental animals intraperitoneally.

2.4 Animals

Male albino mice weighing between 27–30 g was used for this study. The animals were fed Standard mouse cubes and clean drinking water ad libitum. Animals were caged in groups of five. The animals were housed in the Animal House in Kovai Medical Centre and Hospital, College of Pharmacy, Coimbatore.

2.5 Acute Toxicity Tests

The oral acute toxicity of the ethanol extract was estimated in albino mice (27 - 30g) by medium lethal dose (LD_{50}) described by Lorke's method (Lorke, 1983). A total of fifteen albino mice of both sexes were employed, acclimatization period of 24 h was allowed. The extract was weighed and dissolved in distilled water. The test was carried out. In the first, the extract was administered orally at doses of 500, 1000 and 1500 mg/kg to three groups of 5 animals each received respectively. The animals were monitored for 24 h and number of deaths per group recorded. Then, the mice were observed continuously for one hr after the treatment; intermittently for four hrs, and thereafter over a period of 24 hrs (CDER, 1996). The mice were observed for gross behavioural changes such as feeding, hair erection, lacrimation, mortality and other signs of toxicity manifestation (Pillai, 1984). The mice have free access to food and clean water during the experiment.

2.6 Test on early malaria infection (4-day suppressive test)

This test was a modified Makinde *et al.* (1989) and Peters and Robinson (1992) methods. Twenty-five mice were divided into five groups of five mice each were inoculated with the parasite at the commencement of the experiment (day 1). Group's 13 mice received 50, 100 and 200 extract/kg body weight i.p. respectively. While the 4th group which served as the positive control received 5mg of Quinine /kg body weight, mice in 5th group received 1ml distilled water and served as the negative control. On the fifth day (i.e., day 5) two drops of blood samples from the animals' caudal vein were taken and transferred on slides, thus, making thin film from each mouse and staining with Giemsa stain. Then, each stained slide was examined under the microscope with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups so that the average percentage (%) parasitaemia could be evaluated as:

2.7 Test on established infection (curative or Rane test)

The method was a modified-on Ryley and Peters, (1970). Twenty-five mice were divided into five groups of five mice each were inoculated with the parasite on the first day of the experiment (day 1). The mice were not treated until the parasitaemia was established. On day 4 i.e., 72h after the animals were infected. Group's 1-3 mice received 50, 100 and 200mg extract/kg body weight per day for 4 days i.p. While the 4th group which served as the positive control received 4mg of Quinine /kg body weight i.p for, mice in 5th group received 1ml distilled water and served as negative control for the same period. On the fifth day (i.e., day 5) two drops of blood samples from the animals' caudal vein were taken and transferred on slides, thus, making thin film from each mouse and staining with Giemsa stain so that the average percentage (%) parasitaemia could be evaluated for each of the doses using the formula above. After the sixth day, the animals were fed ad libitum and observed for 28 days. Any death that occurred during this period was noted and used to determine the mean survival time.

2.8 Statistical analysis

Data obtained by this study were analysed using SPSS (version 16, 2004). The Student's t-test and ANOVA (one- or two-way) were used to test the differences between groups. Differences between means at 5% level ($P \leq 0.05$) were considered significant.

3. Results

The mortality rate and the acute toxicity symptoms of orally administered *Eichhornia crassipes* extract increased as the dose increased from 0 to 1500 mg/kg (Table 1). The main observed behavioural signs of toxicity were asthenia, piloerection, ataxia, anorexia, urination, diarrhoea, lethargy and coma. There were no signs of toxicity as above said. According to Horn (1956) and Rhiouani *et al*. (2008), plants or plant products

with LD_{50} values higher than 2,000–3,000 mg/kg are considered free of any toxicity. This supports the logical usage of this plant in folk medicine practices. All the treated mice were carefully observed for 24 hours for any signs of toxicity (behavioural changes and mortality). D/T: dead/treated mice; none: no toxic symptoms were recorded during the observation period; latency: time to death (in hours) after the dose administration. Early malaria infection or Peters four days chemo suppressive activity test for the ethanol leaf extract of *Eichhornia crassipes* produced a dose dependent chemo suppression activity and was shown in the table 2. The highest suppression of parasitaemia was observed at the dose of 200mg/kg body weight of mice. Percentage suppression was observed to increase as extract concentration increased. After four days treatment with the different extract doses, the mean parasitaemia of the test groups ranged from 38.0±1.1% to 19.9±0.2 while the corresponding value of the negative control group being 18.7±0.4%. The antimalarial activity produced by the extract was statistically significant ($P < 0.05$) when related to control.

Table 1. Acute oral toxicity of the ethanolic leaf extracts of *Eichhornia crassipes* administered orally to mice

Dose mg/kg	Mortality		Toxic symptoms
	D/T	Latency(h)	
0	0/5	-	None
500	0/5	-	None
1000	0/5	-	None
1500	0/5	-	None

Table 2: Effects of ethanolic leaf extract of *Eichhornia crassipes* on early malaria infection

S.No	Treatment	Doses (mg/kg/day)	*Average parasitaemia in percentage	% chemo suppression	Significance
1.	Extracts	50	38.0±0.1	18.7±0.4	P < 0.05
2.	Extracts	100	28.7±0.5	45.7±0.3	P < 0.05
3.	Extracts	200	19.9±0.2	61.9±0.6	P < 0.05
4.	Quinine	4	1.0±0.0	100	-
5.	Distilled water	1ml	46.7±0.3	0	-

* = Values were presented as Mean ± SEM, n= 5

Table 3: Effects of ethanolic leaf extract of *Eichhornia crassipes* on established malaria infection

S. No	Treatment	Doses (mg/kg/day)	*Average parasitaemia in percentage	% chemo suppression	Significance
1.	Extracts	50	40..8±0.2	26.9±0.2	P < 0.05
2.	Extracts	100	32.8±0.4	31.4±0.5	P < 0.05
3.	Extracts	200	29.3±1.2	43.7±0.4	P < 0.05
4.	Quinine	4	1.0±0.0	100	-
5.	Distilled water	1ml	53.8±0.4	0	-

* = Values were presented as Mean ± SEM, n= 5

Table 4: Phytochemical analysis of ethanolic extract of *Eichhornia crassipes* leaves

Test	Acetone extract	$CHCl_3$ extract	50% Ethanolic Extract	Water extract	Petroleum ether extract	Ethanolic extract
Test for Carbohydrates	-	+	+	+	-	+
Test for proteins	-	+	-	+	+	-
Test for Alkaloids	+	-	-	+	+	+
Test for Flavonoids	-	+	+	+	+	-
Test for Phenols	+	-	+	-	+	+

Test for Tannins	+	-	+	-	+	-
Test for Glycosides	-	-	-	+	-	-
Test for terpenoids	-	-	-	-	-	-
Test for amino acids	+	-	+	+	-	+
Test for starch	-	-	-	-	-	-
Test for sterol	+	-	+	-	+	+
Test for saponins	-	-	+	+	-	+

The result of the *in vivo* evaluation of the *Eichhornia crassipes* extract on established infection showed a slight increase in chemo-suppressive activity. The extract was marginally active at 200 mg/kg per day (43.7%) respectively (Table 3). The mice that received 4mg of Quinine /kg per day however showed 100% chemo suppression. The antimalarial activity produced by the extract was statistically significant ($P < 0.05$) when related to control. The mice that received 4mg of Quinine /kg per day however showed 100% chemo-suppression. Phytochemical screening of the ethanolic extract of the plants revealed that the leaf extract contains terpenoids, flavonoids, alkaloids, saponins, steroids and glycosides. (Table 4)

4. Discussion

This study was undertaken using *in-vivo* model in which the fractions were tested against *P. Falciparum* infected mice. The *in-vivo* model was employed because it considers pro drug effect and possible involvement of immune system in eradication of infection (Waako *et al.,* 2005). Primate models provide a better prediction of evaluation of the efficacy of anti-malaria in human than the rodent models. However, the rodent models have also been validated through the identification of several conventional antimalarials especially with the success of quinine and more recently artemisinin derivatives (David *et al.,* 2004). *In-vivo* murine Plasmodium models such as *P. berghei, P. vinckei* and *P. yoelii* are firmly established models in anti-malarial drug discovery (Petros and Melaku, 2012). *P. berghei* has been used in studying the activity of potential antimalarials in mice (Thomas *et al.,* 1998) and in rats (Pedroni *et al.,* 2006). Therefore, it has been used to predict treatment 54 outcomes and is an appropriate parasite for this study (Gitua *et al.,* 2012; Madara *et al.,* 2012). The chemosuppressive effect by crude leaf extract and solvent fractions of *M. dianthera* against *Plasmodium berghei* compared to the negative control. More significant suppressive effects were noted at 400 mg/kg and 600 mg/kg dose level (Hagazy *et al.,* 2020*).* Since the parasite is sensitive to Quinine, this drug was used as the standard treatment drug in the present study.

The 4-day suppressive test is a standard test commonly used for antimalarial screening (Peter and Anatoli., 1998). It is the most widely used preliminary test, in which the efficacy of a compound is assessed by comparison of blood parasitemia and mouse survival time in treated and untreated mice (Kalra *et al.,* 2006), and the determination of percent suppression of parasitemia is the most reliable parameter (Ene *et al.,* 2008; Peter and Anatoli.,1998). Interestingly, all the fractions at the highest dose significantly prevented weight loss which could be due to appetite enhancing and immunomodulatory components in the fractions (Fentahun *et al*., 2017).

Recently Murugan *et al.* (2015f) reported that antiplasmodial activity of seaweed- synthesized AgNp using the aqueous extract of *Ulva lactuca* against CQ-r and CQ- s strains of *P. falciparum.* IC_{50} of *Ulva lactuca* were 57.26ug ml / and 66.36ug/ml. N. Aarthi and Murugan (2011) reported the antimalarial activity of ethanolic extract of *Phyllanthus niruri* and *Mimosa pudica* against *P. berghi* infecting mice. Furthermore, Bero *et al.* (2013) reported antimalarial activity of *Keetia leucantha* dichloromethane and aqueous twigs extracts assessed in mice at the dose of 200 mg/kg/day. Both extracts exhibited significant effect in inhibiting parasite growth by 56.8% and 53.0% on day 7 post infection. Later on, Ural *et al*. (2014) reported effective *in vivo* antimalarial activity of methanol and water extracts of *Eryngium thorifolium* Boiss (Apiaceae) against *P. berghei* infecting mice. Recently, Rajakumar *et al.* (2015) reported the growth inhibition of *Eclipta prostrata* aqueous leaf extract, palladium acetate, and synthesized PdNP showing promising activity, with IC_{20}, IC_{50}, and IC_{90} values of 1.90, 10.29, and 64.11; 4.49, 9.84, and 23.04; and 4.34, 8.70, and 18.49 mg/kg/body weight, respectively against NK65 strain of *P. berghei*. Chemotherapy has played an important role in the treatment

and control of malaria. Most of the drugs used to treat malaria are quinoline derivatives modeled on the quinine molecule (Krettli *et al.,* 2001). Quinine is still one of the most important drugs for the treatment of uncomplicated malaria, and often the only therapeutic option for the treatment of severe malaria because preparations for intravenous applications are available (Nosten *et al.*, 2006). Generally, a combination of quinine with tetracycline or doxycycline or clindamycin is recommended (Schlitzer, 2008). The most frequently used species of plants were *Azadirachta indica, Senna siamea, Citrus aurantifolia* and *Nauclea latifolia*. As well *Azadirachta indica* has been mostly mentioned as a treatment for malaria in Togo and Kenya (Nguta *et al.,* 2010) and found to have good antiplasmodial activity. A high antimalarial activity was also shown for *Pittosporum viridiflorum* which indicates the interest of Pittosporum genus as a potential source of antimalarials (Ramalhete *et al.*, 2008).

In the qualitative phytochemical analysis, the tested plants showed the presence of various tested groups as shown in table1. It shows that the plant is a rich source of many phytochemicals. Lata *et al.*, 2010 demonstrated the presence of few secondary metabolites in *Eichhornia crassipes* except saponins in aqueous extract. Kandukuri *et al.* (2009) reported the presence of alkaloids, phenols, steroids, tannins and terterpenoids in the methanol extract whereas the authors have reported the absence of flavonoids in water hyancinth. According to the findings of Nataraj *et al* (2009) phenol contents contributes significantly to the total anti-oxidant activity of plants. Humaali *et al* (2009) reported *Eichhornia crassipes* a safe cancer medicine and revealed its tumour inhibition potential. These results of phytochemical screening of study plant were in concurrence with other reports (Thamaraiselvi *et al.,* 2012; Jayanthi and Lalitha, 2013; Aravind *et al.,* 2013). There was a similarity between their results and the obtained results in this current study.

5. Conclusion

The present study is conducted to evaluate the phytochemical, antimalarial activities of *Eichhornia crassipes*. From this study, we concluded that the plant is endowed with many potent phytochemicals like flavonoids, tannins, terpenoids, saponins, cardiac glycosides, quionones and many others. Therefore, we have to exploit the potent possibilities of this plant which possess high therapeutic value and many other uses. The results obtained in the present investigation show the presence of phytochemicals which take part in defense mechanism of the plant. Hence, a complete study conducted with the purpose of finding these chemicals in water hyacinth plant is worthwhile as the output is **"Best out of waste".**

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Compliance with ethical standards

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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