EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
353-360
Year: 2018

ANTIMALARIAL EFFICACY OF *AERVA LANATA* AGAINST CHLOROQUINE SENSITIVE *PLASMODIUM FALCIPARUM* 3D7 STRAIN

P. V. V. Satish[1], D. Santha Kumari[2] and K. Sunita[3]*

[1,2,3]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjunanagar 522510, Guntur, Andhra Pradesh, India.

***Corresponding Author: Dr. K. Sunita**
Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjunanagar 522510, Guntur, Andhra Pradesh, India.

Article Received on 24/12/2017 **Article Revised on 14/01/2018** **Article Accepted on 04/02/2018**

ABSTRACT
The present study aimed to evaluate the antiplasmodial activity of medicinal plant *Aerva lanata* against chloroquine (CQ)-sensitive *Plasmodium falciparum* 3D7 strain and cytotoxicity against THP-1 cell line. The plant *Aerva lanata* was collected from Acharya Nagarjuna University Campus, Nagarjunanagar, Guntur district, Andhra Pradesh, India. Crude extracts from dried leaves, stem and flower of *Aerva lanata* was prepared through soxhlet extraction using methanol, ethyl acetate and chloroform sequentially. These extracts were tested *in vitro* against laboratory adopted *P. falciparum* 3D7 strain. The crude extracts were also tested for their cytotoxicity against THP-1 cell line. The phytochemical screenings were also conducted with standard methods. The IC_{50} values of methanol, ethyl acetate and chloroform extract of leaves, stem and flowers of *A. lanata* showed a range (IC_{50} =15.17 µg/mL - 77.27 µg/mL) of inhibitory concentrations against CQ-sensitive *P. falciparum* strain. The methanol extract of flower (22.60 µg/mL) and ethyl acetate extract of leaves (15.17 µg/mL), flower (48.87 µg/mL) showed good antimalarial activity and were significant at $P < 0.05$ and $P < 0.001$. Among these extracts, the ethyl acetate extract of leaves of *A. lanata* showed excellent antimalarial activity (IC_{50} = 15.17 µg/mL). The methanol and ethyl acetate extract of stem, chloroform extract of leaves showed IC_{50} values between 50-100 µg/mL. The methanol extract of leaves and chloroform extract of stem and flower shown inactive antimalarial activity with IC_{50} values >100 µg/mL. All the extracts were non-toxic to THP-1 cells. The phytochemical screening has revealed the presence of alkaloids, triterpenes, flavonoids, tannins, coumarins, carbohydrates, phenols, saponins, phlobatannins and steroids. It is concluded that the ethyl acetate extract of leaf of *A. lanata* is potent for the development of antimalarial drugs.

KEYWORDS: *Aerva lanata*, antiplasmodial activity, IC_{50}, cytotoxic activity, selectivity index.

INTRODUCTION
Malaria is a curable, preventable and oldest recorded disease can be found even in ancient Indian medical literature like Charaka Samhita. The name malaria was originated from Italian words “mal” and “aria” which means bad air.[1] Malaria is very risky parasitic disease caused by protozoan parasites *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale* and the parasite has transmitted from human to human by the bite of infective *Anopheles* mosquito.[2] Each year 300 to 500 million new cases are being diagnosed and nearly 1.5 million people died; mainstream of deaths reported from Sub Saharan African countries, the majority of them were children under 5 years and pregnant women.[3] Malaria has an enormous impact on child health in malaria endemic countries and contributes to illness, respiratory infection, diarrhoeal disease and malnutrition.[4]

The prevalence of malaria increased in 1980s and 1990s as the parasites developed resistance to the most frequently used antimalarials and the vectors became resistance to insecticides.[5] The first effective drug is chloroquine and its resistance was reported in 1957, consequently distributed all over the world and reported from India in 1976.[6,7] Now artemisinin and its derivates are used as first line treatment according to World Health Organization Proceedings of Malaria Treatment. Unfortunately artemisinin-resistant strains have been reported from Thai- Cambodia in 2009 and hasten the need for new Antimalarial drugs. [8]

Historically and traditionally plant parts have always been used as an important source in the medicine against malaria. About 30% of the world drug sales are based on natural products. It is estimated that there are about 2, 50,000 species of higher plants throughout the world, and most of them have not been examined in detail for

their pharmacological activities.[9] Most effective antimalarial drugs such as chloroquine, quinine and artemisinin are derived from plants. The first effective malarial drug quinine was extracted from Cinchona tree; based on this structure chloroquine and primaquine were synthesized. The other effective drug artemisinin was extracted from Chinese herbal tree *Artemisia annua* in 1972.[10] Artemisinin and its derivatives are now recommended by World Health Organization (WHO) worldwide, in combination with other drugs such as lumefantrine, amodiaquine, mefloquine, sulphadoxine-pyrimethamine (SP) as the first line treatment of malaria.[11] This fact has encouraged the continuing search for new natural product-derived antimalarial drugs. Several plants are used in traditional medicine for the treatment of malaria and fever in malaria endemic areas.[12,13,14]

Aerva lanata Juss. (Amaranthaceae) locally known as 'bui' is an erect, prostrate under shrub and occurs throughout India as a common weed in fields and waste places. Herb, erect or prostrate with a long tap root, branched from near the base; branches many, pubescent or Woolly tomentose, striate. Leaves alternate, 22 × 11.6 cm on the main stem, 610 × 56 mm on the branches, elliptic or obovate, or suborbicular, obtuse or acute, entire, pubescent above, more or less white with cottony hairs beneath; petioles 36 mm long, often obscure. Flowers greenish white, very small, sessile, often bisexual, in small dense subsessile axillary heads or spikes 613 mm long, often closely crowded and forming globose clusters; bracteoles 1.25 mm, long, membranous, broadly ovate, concave, apiculate. Perianth 1.25-1.5 mm long; sepals oblong, obtuse, sometimes apiculate, silkyhairy on the back. Utricle broadly ovoid, acute; stigmas two, seed 0.85 mm in diameter, smooth and polished black.[15]

The plant is diuretic, used in lithiasis. The root is demulcent, diuretic, useful in strangury (slow to be and painful discharge of urine). The roots are used in the treatment of headache. The plant is regarded as a demulcent on the Malabar Coast. It is valued for cough in Ceylon; also as a vermifuge for children. The Meena tribals of the Sawaimadhopur district of Rajasthan give orally the juice of the roots to patients of liver congestion, jaundice, biliousness and dyspepsia. They also give decoction of the whole plant to cure pneumonia, typhoid and other prolonged fevers.[16,17]

Scientific Classification

Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Caryophyllidae
Order: Caryophyllales
Family: Amaranthaceae
Genus: *Aerva*
Species: *A. lanata*

Vernacular Name

The vernacular name of *A. lanata* in language Sanskrit is Astmabyda, in Hindi is Gorkhabundi, in Sindh is Bhui, Punjabi is Buikaltan, in Rajastani is Bhui, in Bengali is Chaya, in Marathi is Kapurmadhura, in Tamil is Sirru pulay vayar, in Malayalam is Cherula, Kannada is Bilesuli and in Telugu is Pindhikura.

Fig. *Aerva lanata* L.

MATERIALS AND METHODS

Plant Collection

Fresh samples of leaves, stem and flower of *Aerva lanata* were collected from ANU campus, Nagarjunanagar of Guntur district, Andhra Pradesh, India. The plant *Aerva lanata* was deposited in the Department of Botany, Acharya Nagarjuna University and voucher specimen was deposited in the department. All the collected plant parts were washed thrice with tap water and twice with distilled water to remove the adhering salts and other Associated organisms. The authentication of the plant species were done by Prof. K. Khasim, Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Extract preparation

Shade-dried plant samples were subjected for in 90% different organic solvents methanol, ethyl acetate and chloroform at 50-60°C in a Soxhlet apparatus. After complete extraction, the filtrates were concentrated separately by rotary vacuum evaporation (>45°C) and then freeze dried (-80°C) to obtain solid residue. The extraction percentage was calculated by using the following formula:

Percentage of extraction

$$= \frac{\text{weight of the extract (g)}}{\text{weight of the plant material (g)}} \times 100$$

The extracts of plant were screened for the presence of phytochemical constituents by following the method of Sofowora (1982) and Kepam (1986).[18,19] The plant extracts were dissolved in dimethyl sulphoxide and filtered through millipore sterile filters (mesh 0.20 µm, Sartorious Stedim Biotech GmbH, Germany). The filtrate was used for testing at different concentrations of 100, 50, 25, 12.5, 6.25 µg/mL.[20]

Parasite cultivation

The antiplasmodial activity of plant extracts was screened against CQ-sensitive *P. falciparum* 3D7 strain obtained from ongoing cultures in the laboratory. They were cultured according to the method of Trager and Jenson (1976)[21] in candle jar desiccator. *P. falciparum* were cultivated in human O^{Rh+} red blood cells using RPMI 1640 medium (Sigma Laboratories Private Limited, Mumbai, India) supplemented with O^{Rh+} serum (10%), 5% sodium bicarbonate and 50 µg/mL of gentamycin sulfate. Hematocrits were adjusted at 2% and cultures of parasite were used when they exhibited 2% parasitemia.[22]

***In vitro* antimalarial screening**

The *P. falciparum* malaria parasite culture suspension of 3D7 (synchronized with 5% sorbitol to ring stage) was seeded (200 µL/well) in 96 well tissue culture plates. Plant extracts were added in µL/well to get different concentrations of extract (100, 50, 25, 12.5, 6.25 µg/mL). Chloroquine treated parasites were kept as positive controls and DMSO treated parasites were kept as negative control. The parasites were cultured for 30 h in candle jar desiccator. The cultures were incubated at 37°C for 48 hours in an atmosphere of 2% O_2, 5% CO_2 and 93% N_2. 18 h before termination of the assay [^{3}H] Hypoxanthine (0.5 µCi/well) was added to each well of 96 well plate. The effects of extracts in the cultures were evaluated by the measurement of [^{3}H] Hypoxanthine incorporation into the parasite nucleic acids.[23] Each treatment has four replicates; at end of experiment one set of the pRBC cells collected from wells and smears were prepared. These smears were fixed in methanol and air dried. The smears were stained with Acradine Orange (AO) stain. Stained smears were observed under UV illumination microscope (Carl Zeiss - Germany) for confirmation of [^{3}H] Hypoxanthine assay data, remaining other three replicates were used for [^{3}H] Hypoxanthine assay. The experiment was terminated and the cultures were frozen and stored in -20^{0}C. The parasites were harvested on glass filter papers using NUNC Cell Harvester and CPM counts were recorded in gamma scintillation counter. Control readings were considered as 100% parasite growth and calculated the parasite inhibition in plant extract treated wells. The parasite inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Average CPM of Control} - \text{Average CPM of plant extracts}}{\text{Average CPM of Control}}$$

The IC_{50} values were determined by plotting concentration of extract on X-axis and percentage of inhibition on Y-axis with dose-response curves using Minitab 11. 12. 32. Bit software.

Cytotoxicity of extracts on THP-1 monocyte cells

The assays were carried out using 96-well flat-bottom tissue-culture plates. Cytotoxic properties of active plant extracts were assessed by functional assay[24] using THP-1 cells. The cells were cultured in RPMI-1640 medium which contained 10% fetal bovine serum, 0.21% sodium bicarbonate (Sigma) and 100 µg/mL penicillin and 50 µg/mL gentamicin (complete medium). Briefly, cells (0.2×10^6 cells/200 µL/well) were seeded into 96-well flat-bottom tissue-culture plates in complete medium. Drug solutions (100, 50, 25, 12.5 and 6.25 µg/mL) were added after 24 h of seeding and incubated for 48 hours in a humidified atmosphere at 37°C and 5% CO_2. DMSO as negative inhibitor ellipticine as a positive inhibitor was added to each well. At the end of experiment ten micro liters of a stock solution of 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 µg/ml in 1x phosphate-buffered saline) was added to each well, gently mixed and incubated for another 4 h. After spinning the plate was centrifuged at 1,500 RPM for 5 min, the supernatant was discarded, subsequently added 100 µL of DMSO (stopping agent). After formation of formazan was read on a micro titer plate reader (Versa max tunable multiwall plate reader) at 570 nm and the percentage of cell viability calculated using the following formula.[25] The selectivity index of *in vitro* toxicity was calculated for each extract as the IC_{50} for THP-1 cells / IC_{50} for *P. falciparum.*

$$\% \text{ Cell viability} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

The IC_{50} values were determined by plotting concentration of extract on X- axis and percentage of cell viability on Y- axis with dose response curves using Minitab 11. 12. 32. Bit software.

Chemical injury to erythrocytes

To evaluate the chemical injury to erythrocytes that might be attributed to the extract. For this, 200 µg/mL of erythrocytes were incubated with 100 µg/mL of the extract, a dose equal to the highest used in the antiplasmodial assay. The conditions of the experiment were continued same as in the case of antiplasmodial assay. After 48 h of incubation, the assay was terminated and thin blood smears were prepared and fixed in methanol and air dried. These smears were stained with Giemsa stain and observed for morphological variations of erythrocytes under high-power light microscope. These morphological findings were compared with the normal erythrocytes of the control group.[26]

RESULTS

The phytochemical studies revealed that the methanol ethyl acetate and chloroform extracts of leaf, stem and flower of *Aerva lanata* have variety of phytochemical constituents namely alkaloids, triterpenes, flavonoids, tannins, coumarins, carbohydrates, phenols, saponins, phlobatannins and steroids as represented in Table 1.

Table 1: Preliminary phytochemical screening of different crude extracts of leaves, stem and flower of *Aerva lanata*

Tested Compound	Leaves			Stem			Flowers		
	ME	EA	CH	ME	EA	CH	ME	EA	CH
Alkaloids	+	+	+	+	+	-	+	+	+
Coumarins	+	-	-	-	+	-	+	+	-
Carbohydrates	-	-	-	-	-	-	-	+	-
Phenols	+	-	-	-	+	-	+	+	-
Saponins	+	-	-	-	+	-	+	-	-
Tannins	-	+	-	+	-	-	+	-	-
Flavanoids	-	+	-	+	-	-	+	-	+
Terpenoids	+	-	-	+	-	-	+	-	-
Phlobatannins	-	-	-	+	+	+	+	-	-
Steroids	-	-	-	-	-	+	-	-	+

Me= Methanol; EtAc= Ethyl Acetate; CH= Chloroform
+ Present, - Absent

In the present study, crude extracts of methanol, ethyl acetate and chloroform, leaves, stem and flowers of *A. lanata* were evaluated for their antimalarial potencies. The IC_{50} values of the tested plant extracts against *P. falciparum* are listed in Table 2. The *in vitro* antiplasmodial activity of biological active substances was categorized into four groups based on IC_{50} value i.e., <5 µg/mL - very active, 5-50 µg/mL - active, 50-100 µg/mL - weakly active, >100 µg/mL inactive.[27]

Based on the above categorization, the IC_{50} value of the methanol, ethyl acetate and chloroform extracts of leaves, stem and flowers of *A. lanata* showed a range (IC_{50} =15.17 µg/mL – 77.27 µg/mL) of inhibitory concentrations against CQ-sensitive *P. falciparum* strain. The methanol extract of flower (22.60 µg/mL) and ethyl acetate extract of leaves (15.17 µg/mL), flower (48.87 µg/mL) showed good antimalarial activity and were significant at $P < 0.05$ and $P < 0.001$. Among these extracts, the ethyl acetate extract of leaves of *A. lanata* showed excellent antimalarial activity (IC_{50} = 15.17 µg/mL). The methanol (IC_{50} = 51.17 µg/mL) and ethyl acetate (IC_{50} = 61.13 µg/mL) extract of stem, chloroform extract of leaves (IC_{50} = 77.27 µg/mL) showed IC_{50} values between 50-100 µg/mL. The methanol extracts of leaves and chloroform extract of stem and flower shown inactive antimalarial activity with IC_{50} values >100 µg/mL. The microscopic observation of inhibition of *P. falciparum* to ethyl acetate extract of leaves (100 µg/mL) of *A. lanata* is shown in Fig. 1.

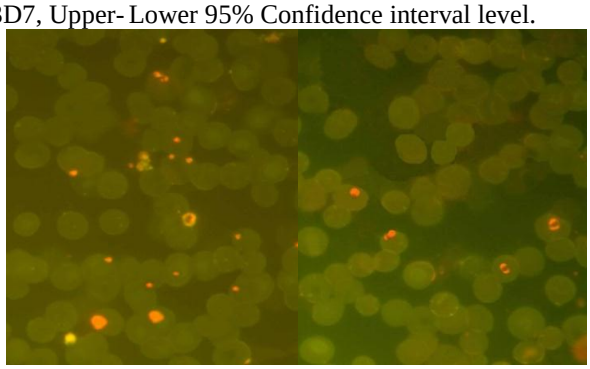
The *in vitro* cytotoxicity studies against THP-1 cell line were conducted for all the extracts. All extracts showed IC_{50} value >20 µg/mL. An extract is classified as non toxic when the IC_{50} value is >20 µg/mL. Based on this, all the plant extracts are not harmful for *in vivo* studies. The selectivity indices indicate the low toxicity of tested extracts and safer for therapies (Table 2).

The microscopic observation of uninfected erythrocytes incubated with the extracts of *A. lanata* and uninfected erythrocytes from the blank column of the 96- well plate showed no morphological differences after 48 h of incubation. Hence, this is the first report of antiplasmodial activity of *A. lanata* against CQ-sensitive *P. falciparum* 3D7 strain.

Table 2: Antiplasmodial activity against CQ-sensitive *P. falciparum* 3D7 strain and cytotoxicity against THP-1 cell line of different crude extracts from *Aerva lanata*.

Plant parts	Crude Extracts	% of Yield	IC_{50} 3D7 strain (µg/mL)(95%*cl*)	IC_{50} THP-1 cells (µg/mL) (95%*cl*)	SI
Leaves	Methanol	2.46	>100	71.33±6.11 (56.16-86.51)	>0.71
	Ethyl Acetate	3.02	15.17±1.04 (12.58-17.75)	>100	>8.21
	Chloroform	4.32	77.27±5.22 (64.31-90.23)	>100	>1.29
Stem	Methanol	19.36	51.57±3.31 (43.35-59.80)	>100	>1.93
	Ethyl Acetate	3.94	61.13±5.54 (47.38-74.89)	75.00±1.00 (72.51-77.48)	1.22
	Chloroform	3.02	>100	>100	>1
Flower	Methanol	5.48	22.60±1.28 (19.43-25.77)	42.00±1.00 (39.51-44.48)	1.90
	Ethyl Acetate	2.46	48.87±3.62 (39.87-57.86)	>100	2.08
	Chloroform	4.32	>100	>100	>1

Values are represented as mean±standard deviation, SI- selectivity index, $SI_{Plasmodium}$ = $IC_{50\ THP-1}$/ IC_{50} *P. falciparum* 3D7, Upper- Lower 95% Confidence interval level.



Control (Positive) Leaf extract treated

Fig 1: Micrograhs of synchronized ring stage *P.f*3D7 culture treated with ethyl acetate extract of leaves (100 mg/µL) of *Aerva lanata* for **48 h showing inhibition of ring stages.**

DISCUSSION

Malaria is still the most dangerous parasitic infectious disease which causes two million deaths every year. It is a great burden to developing nations, a number that could rise due to the increasing multi-drug resistance to all antimalarial drugs currently available.[28] There are several genetic polymorphisms identified in *P. falciparum* and *P. vivax* that can be providing reliable data about the prevalence of drug resistance. Amongst all, the pfcrt, pfmdr1, pfdhfr and pfdhps associated with drug sensitivity, have great role in drug resistance mechanisms in parasites and is directly connected to treatment failure.[29]

From the past 20 years, many strains of *P. falciparum* have become resistant to chloroquine and other anti-malarial drugs. The development and spread of drug resistant strains of *P. falciparum* has limited effectiveness to the currently used malarial drugs. In view of this fact, the emergence and spread of parasites resisting to antimalarial drugs has caused an urgent need for novel effective alternative antimalarial drug compounds to be discovered and developed with minimal side effects.[30]

The discovery of effective antibiotics, drugs, vaccines and other products or methods has decreased the devastating impact of infectious diseases and improved the quality of life. However, the efficacy of many antibiotics and drugs is being threatened by the appearance of pathogen resistance to existing chemotherapeutic agents because of their random and improper use. The use of several antibiotics and drugs is associated with side effects, including allergy, hypersensitivity and immune suppression. Many people who live in developing countries are in lack of the advantages of modern medicine because of its high cost; as a result, the poor people are more prone to infectious diseases. In addition, the prevention and treatment of the infection is difficult due to co-infection with multiple diseases. In favor of all these reasons, presently there is an urgent need to identify new, safe and cost-effective antimicrobial agents that would help to assuage the problems of infectious diseases. Natural products from plants imply an attractive source of antimicrobial agents, for the reason that they are natural and affordable, particularly for rural societies. Acceptance of medicines from such plant derived materials as a choice form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes. Additionally, these compounds may have different mechanisms of action than conventional drugs and could be of clinical importance to improve health care.[31,32]

Plants have proved to be a good source of chemotherapeutic agents over the years. Today, many of the drugs have been derived from plants resources such as quinine, chloroquine and artemisinin. Historically, medicinal plants have provided a source of encouragement for novel therapeutic drugs, as plant-derived medicines have made large contributions. According to the World Health Organization (WHO), at the present time, 80% of the world's population depends on plants for their primary health care. Plants are producing secondary metabolites for their defense, which play an important role of physiological activities in human body. The medicinal value of plants is due to the substances that it contains, which produce a physiological action on the human body. Some examples of these plant substances are alkaloids, essential oils, tannins, resins and many others.[33] India had remarkable biodiversity and rich cultural traditions of plant use. Interestingly, today many of the pharmaceutical companies are utilizing such plant-based formulations in treatment of various diseases and disorders worldwide.[34]

The present investigation was undertaken to evaluate the *in vitro* antiplasmodial activity of plant *Aerva lanata* in different extracts such as methanol, ethyl acetate and chloroform from leaves, stem and flowers along with cytotoxicity. Among the tested extracts, most of the extracts from leaves, stem and flowers have shown maximum antiplasmodial activity due to the synergistic activity of one or more phytochemical constituents except few. Out of the total extracts, the leaf ethyl acetate extracts from *A. lanata* has shown maximum *in vitro* antiplasmodial activity against chloroquine (CQ)-sensitive *P. falciparum* (3D7) strain. According to Rasoanaivo *et al*. (1992), the *in vitro* antiplasmodial activity of the biologically active substances is classified into four groups basing on IC_{50} value (< 5 very active; 5-50 µg/mL active; 50-100 µg/mL weakly active; > 100 µg/mL inactive).

Out of the 9 tested extracts of *A. lanata*, three showed good (IC_{50} ranged from 15.17 µg/mL to 48.87 µg/mL), three exhibited moderate (IC_{50} ranged from 51.57 µg/mL to 77.27 µg/mL) while three shown mild (IC_{50} > 100

µg/mL) activity to *P. falciparum*. The present study has revealed that, the ethyl acetate extract of leaf of *A. lanata* exhibited excellent antiplasmodial activity with IC_{50} value of 15.17 µg/mL and was followed by methanolic extract of flower with IC_{50} value of 22.60 µg/mL. The chloroform extracts of leaves, methanol and ethyl acetate extract of stem of *A. lanata* have shown moderate antiplasmodial activity. The methanol extract of leaves, chloroform extract of stem and flower of *A. lanata* have shown mild antimalarial activity. The IC_{50} value of the ethyl acetate leaf extract of *A. lanata* was quite higher than the positive control of chloroquine and shown active antimalarial activity. Thus, the results of our study are in consistent with the outcome of many researchers who reported the antiplasmodial activity of several plants including polyherbal extracts.[35, 27, 36, 37, 38, 39]

Kaushik *et al*. (2015) has reported promising activity of the ethyl acetate extracts of whole aerial parts of *Aerva lanata* against CQ-sensitive *P. falciparum* 3D7 strain (IC_{50} = 15.4 µg/mL) and CQ-resistant INDO strain (IC_{50} = 8 µg/mL). This is the second report of antiplasmodial activity of *A. lanata* suggesting that *A. lanata* leaves could be of a better source for antiplasmodial molecules than stem-bark and flower since leaves are renewable while girding for stem bark can cause severe injury to the tree. In the present study, the ethyl acetate extract of leaves of this plant was found to have promising antiplasmodial activity against 3D7 strain (IC_{50} = 15.17 µg/mL). The results reported here suggest that leaves may be the better source for antiplasmodial molecules than the stem and flower.[40]

The *in vitro* antiplasmodial activity of the ethyl acetate extract of leaves from *Aerva lanata* may be due to the presence of major chemical classes such as phenols and alkaloids. Hence alkoloids are strong antiplasmodial compounds. Except the alkaloids, the major chemical classes such as coumarins, phenols, polysaccharides and flavonoids also exerted strong antiplasmodial activities.[40] Bandaranayake (2002)[41] reported about the bioactive compounds and chemical constituents of mangrove plants.

Some of the traditional medicine involves the use of crude plant extracts which may contain an extensive diversity of molecules, often with indefinite biological effects. However, most of the available information regarding the medicinal potency of these plants is not provided with credible scientific data. For this reason, several researches have been reported the toxicity of medicinal plants.[33] In the present study, the *in vitro* cytotoxic effect against THP-1 cell lines showed IC_{50} >20 µg/mL. According to Falade *et al*., the ctyotoxicity >20 considered as non toxic to animals and safer for further studies. Thus, maximum the plant extracts are not harmful and safer for therapies.[36]

Similarly, Sree Rekha et al, reported the antimalarial activity of methanol, ethyl acetate and aqueous extracts of *Albezia lebbeck* against CQ-sensitive (3D7) strain of *P. falciparum* and tested for their cytotoxicity on human THP-1 cell line (HEp-2). Out of the 9 test extracts, the ethyl acetate extract of leaf (IC_{50} = 19.22 µg/mL) has shown excellent antimalarial activity. The aqueous extract of leaf and methanol extract of stem were inactive. All the extracts were non toxic to THP-1 cells. They have concluded the leaf ethyl acetate had good antiplasmodial activity (IC_{50} 19.22 µg/mL) with selectivity index ranged >10.52 for THP-1 cells. [42]

The mechanism of action might be due the inhibition of hemozoin biocrystallization by the alkaloids and inhibition of protein synthesis by triterpenoids.[39] Additional *in vitro* and *in vivo* work aimed at understanding the mechanisms of action of the active plant extracts, isolating and characterizing the bioactive constituents is underway in our laboratories and will be reported in due course of time.

CONCLUSION

In conclusion our study shows that the ethyl acetate extract of leaf of *A. lanata* exhibits good *in vitro* antiplasmodial activity against CQ-resistant strain of *P. falciparum.* Further evaluation of the extract may provide potential molecule for therapy of malaria.

ACKNOWLEDGEMENT

The authors are thankful to the Co-ordinator, Department of Zoology and Aquaculture, Acharya Nagarjuna University for providing laboratory facilities.

REFERENCES

1. Guna Rajan K, Balakrishna, Vijayan, Raja S. Evaluation of larvicidal activity of *Pongamia pinnata* extracts against three mosquito vectors. Asian Pac J Trop Biomed, 2013; 3(11): 853-858.
2. John MO, Christopher CK, Tom W, Collins O, Richard O, Zachary L, Daniel O, Jamie L, Stephen M, George A, Alloys S, John M, Sandra S, Richard D, Douglas JP. Parasitemia anemia and malarial anemia in infants and young children in a rural holoendemic *Plasmodium falciparum* transmission area. Am J Trop Med Hyg, 2006; 74(3): 376-385.
3. Tanner M, de Savigny D. Malaria eradication back on the table. Bull World Health Organ, 2008; 86: 82.
4. WHO. The World Health Report. Rolling Back malaria, Making a difference, 1999; 49-61.
5. Le Bars J, Durand R. The mechanisms of resistance to antimalarial drugs in *Plasmodium falciparum*. Fundam Clin Pharmacol, 2003; 17: 147-153.
6. Serena C, Paige ES, Jacques P, Sebastian E, Matthew B, Michael C, David C, Young TC, Mark EH, William A, Julia K, Karine GR. High content live cell imaging for the discovery of new antimalarial marine natural products. BMC Infect Dis, 2012; 12: 1-9.
7. Anupkumar R, Bhawna S, Sharma SK, Ghosh SK, Bhatt RM, Ashwani K, Mohanty SS, Pillai CR, Dash AP, Neena V. *In vitro* assessment of drug resistance

in *Plasmodium falciparum* in five States of India. Indian J Med Res, 2012; 135: 494-499.
8. Noedl H, Youry Se, Schaecher K, Smith BL, Sochet D, Fukuda MM. Evidence of artemisinin-resistant malaria in Western Combodia. N Engl J Med, 2008; 359: 2619-2620.
9. Bobby MN, Wesely EG, Johnson MA. In vitro anti-bacterial activity of leaves extracts of *Albizia lebbeck* Benth against some selected pathogens Asian Pac J Trop Biomed, 2012; S859-S862.
10. White NJ. Qinghaosu (Artemisinin): The price success. Science, 2008; 320: 2619–2620.
11. Mutabingwa TK. Artemisinin –based combination therapies (ACTs): best hope for malaria treatment but inaccessible to the needy! Acta Trop, 2005; 95: 305–315.
12. P.V.V. Satish and K. Sunita. *In vitro* antiplasmodial efficacy of mangrove plant, *Ipomoea pes-caprae* against *Plasmodium falciparaum* (3D7 strain). *Asian Pacific J of Trol Dis.*, 2015; (12): 947-956.
13. P.V.V. Satish, K. Somaiah, P. Brahmam, N. Sree Rekha and K. Sunita. Antimalarial activity of *Prosopis cineraria* (L) Druce against chloroquine sensitive *Plasmodium falciparum* 3D7 strain. *European Journal of Pharmaceutical and Medical Research*, 2015; 2(7): 295-303.
14. P.V.V. Satish, K. Somaiah, P. Brahmam, N. Sree Rekha and K. Sunita. 2015. Cytotoxicity evaluation of *Prosopis cineraria* (L) Druce against brine shrimp. *European Journal of Biomedical and Pharmaceutical Sciences*, 2015; 3(1): 201-207.
15. Kirtikar KP, Basu BD, Mahaskar C. Indian Medicinal Plants (2nd Edn.). International Book Distributors, Allahabad., 1987; p 2051.
16. Sharma A, Sharma SC, Vaghela JS. Phytopharmacological investigation of *Aerva lanata* flowers with special emphasis on diuretic activity. Pharmacogn J, 2010; 2: 59-62.
17. Manoj G, Anil P, Nagori BP, Sasmal D. *Aerva lanata*: A review on phytochemistry and pharmacological aspects. Pharmacogn Rev, 2011; 5(10): 195-198.
18. Sofowora A. Medicinal plants and traditional medicine in Africa. John Wiley and Sons, New York, 1982; 251.
19. Kepam W. Qualitative organic analysis (Spectrochemical techniques). EdII. McGraw Hill, London, 1986; 40-58.
20. Ouattara Y, Sanon S, Traore Y, Mahiou V, Azas N, Sawadogo L. Antimalarial activity of *Swartzia madagascariensis* desv. (leguminosae), *Combretum glutinosum* guill. and perr. (Combretaceae) and *Tinospora bakis* miers. (menispermaceae), Burkina Faso medicinal plants. Afr J Tradit Complement Altern Med, 2006; 3(1): 75–81.
21. Trager W, Jenson JB. Human malaria parasites in continuous culture. Science, 193: 673-675.
22. Trager W. The cultivation of *Plasmodium falciparum*: applications in basic and applied research in malaria. Ann Trop Med Parasitol, 1987; 81: 511–529.
23. Simonsen HT, Jesper BN, Ulla WS, Ulf N, Pushpagadan P, Prabhakar J. *In vitro* screening of Indian medicinal plants for antiplasmodial activity. J Ethnopharacol, 2001; 74: 195–204.
24. Basim MA, Abdalla AA, Faris DM. *In vitro* inhibition of human leukemia THP-1 cells by *Origanum syriacum* L. and Thymus vulgaris L. extracts. BMC Research Notes, 2014; 7: 612-618.
25. Khonkarn R, Okonogi S, Ampasavate C, Anuchapreeda S. Investigation of fruit peel extracts as sources for compounds with antioxidang and antiproliferative activities against human cell lines. Food Chem Toxicol, 2010; 48: 2122-2129.
26. Ravikumar S, Samuel JI, Palavesam S, Murugesan G. *In vitro* antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive *Plasmodium falciparum*. Parasitol Res, 2011; 108: 873–878.
27. Rasoanaivo P, Ratsimamanga, Urverg S, Ramanitrhasimbola D, Rafatro H, Rakoto Ratsimamanga A. Criblage d'extraits de plantes de Madagascar pour recherche d'activite antipaludique et d'effet potentialisateur de la chloroquine. J. Ethnopharmacol, 1992; 64: 117–126.
28. Jovel IT, Rosa EM, Engels B, Rita P, Jackeline A, Gustavo F, Pedro EF, Maria IV, Irma GE, Anders B, Johan U 2011. Drug resistance associated genetic polymorphisms in *Plasmodium falciparum* and *Plasmodium vivax* collected in Honduras, Central America. Malar J, 2011; 10: 376.
29. Pascual A, Philippe P, Françoise BV, Fabrice S, Denis M, Stéphane P, Pascal D, Didier B, Danièle M, Bernard F, Guillaume M, Nathalie B, Claude O, Eric D, Christophe R, Bruno P. Ex vivo activity of the ACT new components pyronaridine and piperaquine in comparison with conventional ACT drugs against isolates of *Plasmodium falciparum*. Malar J, 2012; 11: 45. doi: 10.1186/1475-2875-11-45.
30. Okigbo RN, Ramesh P. Effects of plants and medicinal plant combinations as anti-infectives. Af J Pharmacy and Pharmacol, 2008; 2(7): 130–135.
31. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement Altern Med, 2014; 14(7): 79-89.
32. Yen WJ. Possible anti-obesity therapeutics from nature-A review. Phytochem, 2010; 71: 1625-1641.
33. Lilybeth FO, Olga MN. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan city, Philippines. Int Res J Biologcal Sciences, 2013; 2(11): 74–77.
34. Johnson M, Babu A, Irudayaraj V. Antibacterial studies on *in vitro* derived calli of *Ocimum basilicum* L. J Chem Pharm Res, 2011; 2(2): 298–305.

35. Chenniappan K, Kadarkarai M. *In vitro* antimalarial activity of traditionally used Western Ghats plants from India and their interactions with chloroquine against chloroquine-resistant *Plasmodium falciparum*. Parasitol Res, 2010; 107(6): 1351-1364.
36. Falade MO, Akinboye DO, Gbotosho GO, Ajaiyeoba EO, Happi TC, Abiodun OO, Oduola AM. *In vitro* and *in vivo* antimalarial activity of *Ficus thonningii* Blume (Moraceae) and *Lophira alata* Banks (Ochnaceae), identified from the ethnomedicine of the Nigerian Middle Belt. Parasitol, 2014; 97: 28-53.
37. Pothula VVS, Sunita K. *In vitro* antiplasmodial efficacy of mangrove plant, *Ipomoea pes-caprae* against *Plasmodium falciparaum* (3D7 strain). Asian Pac J Tro Dis, 2015; 5(12): 947-956.
38. Satish PVV, Sunita K. *In vitro* and *in vivo* antimalarial efficacy of *Pongamia pinnata* (L) Pierre against *Plasmodium falciparum* (3D7 Strain) and *Plasmodium berghei* (ANKA). BMC Compl Alter Med, 2017; 17: 458.
39. Satish PVV, Santha Kumari D, Sree Rekha N, Sunita K. *In vitro* and *in vivo* antimalarial screening of *Calotropis gigantea* against *Plasmodium falciparum* (3D7 Strain) and *Plasmodium berghei* (ANKA) along with cytotoxicity. Ind J of Vec Bor Dis, 2017; 53(4): 215-225.
40. Sherman PW, Billing J. Darwinian gastronomy: why we use spices. Bioscience, 1999; 49: 453–463.
41. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol Manag, 2002; 10: 421–452.
42. N. Sree Rekha, P.V.V. Satish, K. Somaiah, P. Brahmam and K. Sunita 2016. Antimalarial activity of *Albizia lebbeck* Benth against chloroquine sensitive *Plasmodium falciparum* 3D7 strain. *European Journal of Biomedical and Pharmaceutical Sciences*, 2016; 3(1): 271-276.
43. Samuel JI, Ravikumar S, Suganthi P. *In vitro* antiplasmodial effect of ethanolic extracts of costal medicinal plants along Palak Strait against *Plasmodium falciparum*. Asian Pac J Biomed, 2012; 2(5): 364-367.