Contents lists available at www.innovativejournal.in

INNOVATIVE JOURNAL OF MEDICAL AND HEALTH SCIENCE

Journal homepage: http://innovativejournal.in/ijmhs/index.php/ijmhs

ISSN No. 2277-4939

IN VITRO ANTIPLASMODIAL ACTIVITY OF KANI HERB *ALSTONIA SCHOLARIS* AGAINST *PLASMODIUM FALCIPARUM*

C. Christina[1], S. Prasanna kumar[2], J. Margret beula[1], N. Chandra Lekha[1], N. Jeyaraj[2], S. Ravikumar[2*]

[1]PG & Research Department of Chemistry, Scoot Christian College, Nagercoil – 629003, Tamil Nadu, India.
[2] Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi -623409, Tamil Nadu, India.

ARTICLE INFO

Corresponding Author:
S. Ravikumar, Professor,
Department of Oceanography and Coastal Area Studies
School of Marine Sciences
Alagappa University
Thondi campus
Ramanathapuram Dist
Tamil Nadu, India.

Key words: Antiplasmodial activity, *Plasmodium falciparum, Alstonia scholaris*, Kani herb.

DOI:http://dx.doi.org/10.15520/ijmhs.2015.vol5.iss4.81.166-169

ABSTRACT

Background: The *Alstonia scholaris* plant grows throughout India, in deciduous and evergreen forests and also in plains. The plant is known to possess a lot of medicinal properties in folk medicine by the Kani tribals in Kanyakumari district. *A. scholaris* bark has been as one of the ingredient used in the marketed Ayurveda preparation Ayush-64, NRDC, India. The milky juice of the plant is applied on wounds, ulcers and rheumatic pains. Tincture of the bark and juice of the leaves act as powerful galactogogue in certain cases. The drug is also used in case of snake bite. **Objectives:** The present study has been made an attempt to find out the antiplasmodial activity of different plant parts (Leaf, root, bark, stem bark and fruit) of *A. scholaris* against the most prevalent Indian malarial Parasite *P. falciparum.* **Methods:** Different plant arts Viz., Leaf, stem, bark, root and fruit of *A. scholaris* plant was collected from SouthWest coast of India, Kanyakumari, Tamil Nadu during monsoon and non-monsoon months. The plant parts were cut into pieces and kept for shade drying. Moisture free samples were subjected for percolation by soaking it in ethanol: water mixture (3:1 ratio). Different concentrations of filter-sterilized crude extract from plants (100, 50, 25, 12.5, 6.25 and 3.125 µg. ml^{-1}) was incorporated into 96-well tissue culture plate containing 200 µl of *P. falciparum* culture with fresh red blood cells diluted to 2% haematocrit. **Results:** Among the plant parts tested against *P. falciparum,* the bark and leaf extract of *A. scholaris* collected during non-monsoon season exhibited IC_{50}<3.125 µg. ml^{-1}; followed by the stem extract (IC_{50} 3.125 µg. ml^{-1}). **Conclusion:** It can be concluded from the present study that the bark and leaf extracts of *A. scholaris* potentiate the antiplasmodial activity against *P. falciparum.*

INTRODUCTION

The plant, *Alstonia scholaris*, invites attention of the researchers worldwide for its pharmacological activities ranging from antimalarial to anticancer activities. The plant of *Alstonia scholaris* (L.) R. Br. belongs to family Apocynaceae and is also known as Devil's tree or Dita Bark tree or Saptaparni evergreen tree or shrubs with white funnel shaped flowers and milky sap. *Alstonia scholaris* is a small tree that grows up to 40 m tall and is glabrous. The bark is grayish; branhlets are copiously lenticellate. The upperside of the leaves are glossy, while the underside is grayish. Leaves occur in whorls of 3-10; petioles are 1-3 cm; the leathery leaves are narrowly obovate to very narrowly spathulate, base cuneate, apex usually rounded; lateral veins occur in 25-50 pairs, at 80-90° to midvein. Cymes are dense and pubescent; peduncle is 4-7 cm long. Pedicles are usually as long as or shorter than calyx. The corolla is white and tube-like , 6-10 mm; lobes are broadly ovate and broadly obovate , 2-4.5 mm, overlapping to the left. The ovaries are distinct and linear. Flowers bloom in the month October. The flowers are very fragrant similar to the flower of *Cestrum nocturnum.* Seed of A. scholaris are oblong, with ciliated margins, and ends with tufts of hairs 1.5-2 cm. the bark is almost odourless and very bitter, with abundant bitter and milky. In India, The bark of *Alstonia scholaris* is used solely for medicinal purpose, ranging from malaria and epilepsy to skin conditions and asthma. In ayurvedha it is used as a bitter and astringent herb for treating skin disorders, malarial fever, urticaria, chronic dysentery, diarrhea, in snake bite and for upper purification process of Panchakarma. The milky juice of the tree is applied to ulcers. The bark contains the alkaloids ditamine, echitenine and echitamine and used to serve as an alternative to quinine. At one time, a decoction of the bark was used to treat diarrhea and malaria, as a tonic, febrifuge, emmenagogue, anticholeric and vulnerary. A decoction of the leaves was used for beriberi. Ayurveda recommends *A. scholaris* for bowel complisnts. In Sri Lanka its light wood is used for coffins. In borneo the wood close to the root is very light and white colour, and used for net floats, household utensils, trenchers, corks, etc. All these

facts were freezed by the Kanikars due to ethical sanctity and religious belief. Scientifically, the extract prepared from the plant has been reported to possess cytotoxic activity. The active compounds include alkaloids, flavonoids, etc. These are present in all parts of the plant. An ethanol extract of the bark of Alstonia scholaris enhanced the anticancer activity of berberine in the ehrlich ascites carcinoma-bearing mice. This extracts also showed cytotoxic activity to HeLa cells. It contains echitamine and loganin as major compounds and could potentially be used as anti-irrigation agent. Malaria still kills nearly a million people worldwide each year, and most malarial deaths are due to Plasmodium falciparum (WHO 2009). But so far attempts has not been initiated for the scientific evaluation of the antimalarial activity and hence the present study has been undertaken to find out the antiplasmodial potential *A. Scholaris* plant parts extracts against *P. falciparum.*

METHODOLOGY

Collection of plants

Different plant arts *Viz.,* Leaf, stem, bark, root and fruit of *A. scholaris* plant was collected from SouthWest coast of India, Kanyakumari, Tamil Nadu during monsoon and non-monsoon months. The collected samples were washed thrice with tap water and twice with distilled water to remove the adhering associated animals. Voucher specimen was deposited in the herbarium facility (sponsored by the Indian Council of Medical Research, New Delhi) maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Tamil Nadu, India.

Extraction of bioactive principles

The plant parts were cut into pieces and kept for shade drying. Moisture free samples were subjected for percolation by soaking it in ethanol: water mixture (3:1 ratio). After 21 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporator (>45°C) and then freeze dried (-80°C) to obtain solvent free solid residue. The extracts were dissolved in dimethyl sulphoxide (Hi media Laboratories Private Limited, Mumbai, India) and filtered through sterile millipore filters (mesh 0.20 µm, Sartorious Stedim Biotech GmbH, Germany). The filtrate was used for testing at different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg.ml^{-1}) (Jacob inbaneson *et al.,* 2012).

Culture maintenance

The *in vitro* antiplasmodial activity of extracts from *A. scholaris* was assessed against *P. falciparum* (obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Indian Institute of Science, Bangalore, India). *P. falciparum* were cultivated in human O Rh$^+$ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India) (Moore *et al.,* 1967) supplemented with O Rh$^+$ serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, India) and 40 µg. ml^{-1} of gentamycin sulphate (HiMedia Laboratories Private Limited, Mumbai, India). Haematocrits were adjusted at 5% and parasite cultures were used when they exhibit 2% parasitaemia (Trager, 1987).

In vitro antiplasmodial activity

Different concentrations of filter-sterilized crude extract from plants (100, 50, 25, 12.5, 6.25 and 3.125 µg. ml^{-1}) was incorporated into 96-well tissue culture plate containing 200 µl of *P. falciparum* culture with fresh red blood cells diluted to 2% haematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% haematocrits and positive control was maintained with parasitized blood culture treated with Artemether and chloroquine (Azas *et al.,* 2002). Parasitaemia was evaluated after 24 h and 48 h by giemsa stain and the average percentage suppression of parasitaemia was calculated by the following formula: average % suppression of parasitaemia= average % parasitaemia in control−average % parasitaemia in test/average % parasitaemia in control×100.

ANTIPLASMODIAL ACTIVITY CALCULATION AND ANALYSIS

The antiplasmodial activity of plant extracts expressed by the inhibitory concentrations (IC_{50}) of the drug that induced 50% reduction in parasitaemia compared to the control (100% parasitaemia). The IC_{50} values were calculated (Concentration of extract in the X-axis and percentage of inhibition in the Y - axis) using office XP (SDAS) software. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al.,* (1992) and suggested that, an extract is very active if IC_{50}< 5 µg. ml^{-1}, active IC_{50} < 50 µg. ml^{-1}, weakly active IC50< 100 µg. ml^{-1} and inactive IC_{50} > 100 µg. ml^{-1}.

CHEMICAL INJURY TO ERYTHROCYTES

To assess any chemical injury to erythrocytes that might attributed by the extract, 200 µl of erythrocytes was incubated with 100 µg. ml^{-1} of the extract at a dose equal to the highest used in the antiplasmodial assay. The conditions of the experiment were maintained as in the case of antiplasmodial assay. After 48 h of incubation, thin blood smears were stained with giemsa stain and observed for morphological changes under high-power light microscope. The morphological findings were compared with those erythrocytes that were uninfected and not exposed to extract (Waako *et al.,* 2007).

RESULTS AND DISCUSSION

Among the plant parts of *A. scholaris* tested against *P. falciparum* the bark extracts collected from monsoonal months exhibited maximum activity of <3.125 µg. ml^{-1}; followed by the leaf extract collected during non-monsoon season (IC_{50} < 3.125 µg. ml^{-1}) at 48 hrs of incubations. The bark extract collected from non-monsoonal month showed maximum activity of 3.125 µg. ml^{-1}; followed by the leaf extract collected during monsoonal month (IC_{50} 11.35 µg. ml^{-1}) at 48 hrs of incubations (**Table 2**). Plants plays a vital role in maintaining human health and improving the quality of human life from thousands of years and serves to human the valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine contains natural substances that can promote health and reduce illness. The plant of *Alstonia scholaris* (L.) R. Br. belongs to family Apocynaceae and is also known as Devil's tree or Dita Bark tree. The plant grows throughout India, in deciduous and evergreen forests and also in plains. This plant parts has been showed a variety of pharmacological activities. The bark is official in the Indian, British and French Pharmacopoeias. The plant has been reported for anticancerous (Kamarajan *et al*., 1991; Saraswathi *et al*., 1997,1998,1999; Keawpradub *et al*., 1997; Jagetia *et al*., 2003; 2005; Jagetia and Baliga, 2003a,b; 2004; 2005; 2006; Nersesyan *et al*., 2004; Baliga, 2010; Jahan *et al*., 2009a; Jain *et al*., 2009c); antipsychotic (Campos *et al*., 1999, 2004a; de Moura Linck *et al*., 2008); antidiabetic and antihyperlipidemic (Arulmozhi *et al*., 2010b; Bandawane *et al*., 2011); antiplasmodial (Keawpradub *et al*., 1999); antimalarial (Gandhi and Vinayak, 1990); larvicidal

(Kaushik and Saini, 2009) activities; Considering these great features the present study has been made an attempt to find out the antiplasmodial activity of *A. scholaris* against *P. falciparum*. In the present study, among the plant parts tested the bark extracts exhibited maximum activity (<3.125 µg. ml^{-1} and 3.125 µg. ml^{-1}) against *P. falciparum;* followed by the leaf extracts exhibited maximum activity (<3.125 µg. ml^{-1} and 11.35 µg. ml^{-1}). Similarly Keawpradub *et al*., (1999) evaluated the antiplasmodial activity of the methanolic extracts of various parts of *A. scholaris* which was tested against multidrug resistant K1 strain of *Plasmodium falciparum* cultured in 73 human erythrocytes. Pronounced antiplasmodial activity was exhibited. The indole alkaloids were isolated from the active extract and were subsequently tested against the K1 strain of P. falciparum. They reported pronounced antiplasmodial activity mainly among the bisindole alkaloids, particularly villalstonine and macrocarpamine with IC50 values of 0.27 and 0.36 µM, respectively. Other alkaloids like Corialstonine and corialstonidine, obtained *A. scholaris*, are also found to be active against *P. falciparum* (Jegetia, 2005). The antiplasmodial activity was tested for plant *Alstonia boonei*. The antimalarial activity was found to reside predominantly in N- Hexane and Chloroform fractions. The crude ethanol extracts of the stem bark of Alstonia boonei inhibited the schizont of *Plasmodium falciparum*. It is reported to contain various alkaloids, flavonoids and phenolic acids. Alstonia scholaris is known to possess in vitro antioxidant, free radical scavenging, analgesic, anti-inflammatory and anti-ulcerogenic activities, anti-anxiety and anti-depressant activities. The bark extracts of Alstonia scholaris possess immunostimulating effect [Iwo *et al*., 2000]. The plant is known to possess anticancer activity on skin carcinogenesis in mice and cytotoxic activity to HeLa cells (Jahan *et al*., 2009). The combination of Alstonia scholaris and berberine hydrochloride, a topoisomerase inhibitor showed enhanced chemomodulatory activity in Ehrlich ascites carcinoma-bearing mice (Jagetia *et al*., 2004). The leaves of A. scholaris possess broncho-vasodilatory activity (Channa *et al*., 2005). Acetone extract of A. scholaris possesses schizonticidal properties. Methanolic crude extract possesses Anti-diarrhoeal and spasmolytic activity (Shah *et al*., 2010). The alkaloid fraction of the leaves showed anti-tussive, anti-asthmatic and expectorant activities and is proved to be a valuable lead material for respiratory diseases drug development (Shang *et al*., 2010). So, it can be concluded from the present study that, the bark and leaf crude extracts of *A. scholaris* potentiate antiplasmodial activity and possess lead compounds for the improvement of safe and efficacious antiplasmodial drugs.

Table 1 Suppression of Parasitemia (IC_{50}) of *A. scholaris* plant extract at 24 hrs against *P. falciparum*

Sample	Monsoon	Non-monsoon
Bark	14.26	<3.125
Root	56.40	72.39
Leaf	22.98	3.125
Fruit	N_fN_t	76.26
Stem	40.42	47.20
Positive controls Chloroquine	<3.125	<3.125
Artemether	<3125	<3.125

N_fN_t – No fruit not tested

Table 2 Suppression of Parasitemia (IC_{50}) of *A. scholaris* plant extract at 48 hrs against *P. falciparum*

Sample	Monsoon	Non-monsoon
Bark	<3.125	3.125
Root	34.60	66.09
Leaf	11.35	<3.125
Fruit	-	70.45
Stem	22.50	45.75
Positive controls Chloroquine	<3.125	<3.125
Artemether	<3125	<3.125

N_fN_t – No fruit not tested

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of Alagappa University for providing required facilities.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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How to cite this article: N. JEYARAJ, S. RAVIKUMAR, C. Christina, S. Prasanna kumar J. Margret beula, N. Chandra Lekha. In vitro antiplasmodial activity of Kani herb Alstonia scholaris against Plasmodium falciparum. **Innovative Journal of Medical and Health Science**, [S.l.], v. 5, n. 4, p. 166-169, aug. 2015. ISSN 2277-4939. Available at: <http://innovativejournal.in/ijmhs/index.php/ijmhs/article/view/81>. Date accessed: 02 Sep. 2015. doi:10.15520/ijmhs.2015.vol5.iss4.81.166-169.