

# Night Jasmine (*Nyctanthes arbortristis*) having *in vitro* antimalarial activity from the leaves – a confirmation from ethnobotanical studies and traditional uses

Dr. Debasis Bisoi<sup>1</sup>, Dr. Ashok Kumar Panigrahi<sup>2</sup>, Dr. Lopamudra Das<sup>3</sup>

<sup>1</sup> MD (Pharmacology), DM (Clinical Pharmacology), Assistant Professor, Department of Pharmacology, All India Institute of Medical Sciences, Bibinagar, India (corresponding author);

<sup>2</sup> MD (Pharmacology), Associate Professor, Department of Pharmacology, V.S.S. Medical College, Burla (VIMSAR) Sambalpur, India;

<sup>3</sup> MSc. (Botany), PhD, Assistant Professor, Department of Botany, J.K.B.K. Government College, OMP Square, Cuttack, India

<sup>1</sup>Email: bisoidebasis@gmail.com

## ABSTRACT:

**Background:** Night jasmine (*Nyctanthes arbortristis* or NAT) is a sacred plant (known as Parijat in Sanskrit) and commonly used traditional medicine. It was observed that the leaves of the plant are used in treating malaria. The present work has made an attempt to make ethnobotanical studies, isolation of the phytochemical constituents and mechanism of the pharmacological actions and therapeutic uses with focus on malaria. **Objectives:** (1) Identify the plant material for use in traditional medicine. (2) Gather laboratory evidence that the chemical constituents are comparable to standard treatment in malaria.

**Methods:** The leaves of the plant (NAT) were identified and the sample specimens were kept in herbarium after authentication. The phytoconstituents were identified by column chromatography. The antimalarial activities were studied. The *in vitro* activity of leaves of NAT was compared with chloroquine (CQ).

**Results:** Phytochemical screening of ethanolic extract of NAT leaves revealed the presence of saponins, alkaloids, terpenes, flavonoids and glycosides. The leaf extract showed antimalarial action (IC<sub>50</sub> of NAT 24.92 µg/ml and IC<sub>50</sub> of chloroquine 21.73 µg/ml) [P<0.05] which was comparable to chloroquine. Earlier two studies showed the antimalarial action of the leaf paste having pharmacological actions in the clinical studies. It appeared likely that the iridoid glycosides present in the ethanolic extract caused the pharmacological action. Further the pharmacological actions might be like that of artemisinin in producing oxidative stress. It may have a future use in combination therapy with artemisinin

**Conclusions:** *The present study justified the ethnobotanical use of Night Jasmine in malaria. The chemical constituents attributable to pharmacological action might be due to iridoid glycosides which produced oxidative stress.*

**Key words:** *Iridoid glycosides, Malaria, Night jasmine, Nyctanthes arbortristis, oxidative stress*

## INTRODUCTION:

Malaria is a global health problem caused by six species of malaria parasites<sup>1</sup>. Though malarial deaths have been reduced, still it is a global burden of disease of tropical and sub tropical regions. More than 200 million cases and 445000 deaths have been reported in the year 2016<sup>2</sup>. The malaria caused by *Plasmodium falciparum* in great majority occurs in Sub-Saharan Africa<sup>3</sup>. The clinical manifestations are presented as irregular fever with rigor and chills, hepatosplenomegaly, anemia and may be more susceptible to cause death in pregnant women and children<sup>4-5</sup>. Unfortunately, there is no vaccine for malaria and the drugs are only measures for prophylaxis and therapy.<sup>6-8</sup>

Traditionally plants have been used for the treatment of malaria. The bark of Cinchona (Sweet bark or Peruvian bark) had been traditionally used by the indigenous Peruvians to treat shivering. By 1640 Cinchona was used to treat fevers and the powder of Cinchona bark was given as beverages cured the “fevers and tertians” The bark contains Quinine- an alkaloid which is used for the treatment of malaria.<sup>9</sup>

Artemisinin, a sesquiterpene lactone obtained from Chinese herbal drug “qinghao” from *Artemisia annua* has been the first line medicine of value for more than 2000 years. In 1972, Chinese scientist had extracted artemisinin, the major antimalarial ingredient. The water soluble hemisuccinate ester of dihydroartemisinin commonly known as artesunate is commonly used as antimalarial.<sup>10-11</sup>

Described as “a sacred plant” from India, Night Jasmine or *Nyctanthes arbortristis* (NAT) has been quoted in the classical texts of Chakradutta for “Visham Jwara” (malaria) and is used in the treatment of Ayurvedic line of treatment.<sup>12</sup> In Sanskrit, the plant is known as Parijat and known for its many medicinal properties.<sup>13</sup> Night Jasmine (NAT) is recognised in many local languages as Harsingar, Shefali, Gangasiuli etc.<sup>13-16</sup> The plant is used in various systems of medicines and also by tribal people of various parts of India for a wide range of diseases.<sup>13-16</sup> Presently, there is an interest in research on Night Jasmine plant (NAT) in general and for malaria in particular.<sup>17-23</sup>

Malaria is very common in India except 5 out of all the states where prevalence is high. Nearly 70% of these malaria cases are reported from Odisha (36%), Chhatisgarh (12%), Jharkhand (9%), Madhya Pradesh (9%) and Maharastra (5%).<sup>25</sup> It has been observed that the antimalarial potential of traditionally used plant, the Night Jasmine (NAT), has been explored by reverse pharmacology approach. The trend of “bench top to bedside” approach is reversed as “bedside to bench” approach.<sup>26-27</sup> The early clinical observation was made in 92 of 120 patients (76.77%) of malaria by giving the paste of fresh leaves of Night Jasmine for 7-10 days<sup>26</sup>. In a similar study the plant (leaves) showed in 10 out of 20 patients antimalarial parasite clearance and subsiding of fever.<sup>27</sup>

It was observed and hypothesized by one of the authors that the malaria can be treated by the leaves of Night Jasmine (NAT). Although occasionally caused vomiting, the plant medicine was safe, tolerable and effective by causing patient wellbeing by subsiding the irregular fever. This led to his guiding an MD (Pharmacology) dissertation in VSS Medical College, Burla, Odisha from the year 2010 to 2013. The present work has made an attempt to make ethnobotanical studies, isolation of phytochemical constituents and mechanisms of pharmacological basis of their therapeutic applications especially in malaria. The present study made efforts to make conformational study of

traditional use of NAT in malaria by doing reverse pharmacology. Further the authors also searched whether similar plants have similar potential to treat malaria or other ailments.

### Objectives of the Study:

1. To identify the plant material based on traditional use of medicines from Night Jasmine (NAT)
2. To gather laboratory evidence (by doing reverse pharmacology) that the chemical constituents are comparable to standard treatment in malaria.

### MATERIAL AND METHODS:

The present study was done after approval from Intuitional Ethics Committee of V.S.S. Medical College, Burla.

### Plant Materials:

Night jasmine (*Nyctanthes arbortristis*) is known as Parijat and is known in many names in local languages.<sup>13-16</sup> The various parts of the plant are useful in many diseases. The plant is widely distributed/ cultivated in various regions of tropics and subtropics. The plant has an average life span of 10 years and grows as a shrub. It belongs to *Oleaceae* family and the flowers have a smell like jasmine which blooms in the night. The corolla contains a colouring material called Nyctanthin<sup>14-16</sup> and iridoid glycosides<sup>17</sup>. Leaves are hairy green and rough. It measures about 5-10 cm long and 2.5 - 5 cm wide. The margin is serrated and oval shaped with an acute apex. The phytoconstituents of the leaves are reported to contain iridoid glycoside along with many others.<sup>15-17</sup>

Fresh leaves of *Nyctanthes arbortristis* (NAT) were collected from Rairakhol Forest, Sambalpur, India during the months of October 2011. They were identified and authenticated by Dr. Pratap Panda, PhD. Regional Plant Research Centre (RPRC), Bhubaneswar, India. The sample specimen was kept in the herbarium vide voucher number 9033 for future reference. Dry powder of leaves was made, weighed and extraction was done in 95% ethanol. NAT powder 140 gram was dissolved in 400 ml. of the solvent for 72 hours at room temperature and then heated at 100°C in the apparatus. The extract was collected when volume reduced to 200 ml. and then evaporated to dryness in water bath at 40-50°C. The final extracts yield was 4.26% w/w of the NAT powder. Each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator. The dried extract was stored in the refrigerator at 4°C.

### Phytochemical screening

This was done by column chromatography method. For the fractionation of the extract a glass column (30cm length 2 cm diameter) was loaded by means of slurry packing. A beaker was half-filled with silica (Silica gel 60. Size 0.08mm, Merck, Darmstadt) and dichloromethane added to obtain a pourable slurry. The column was left to settle for at least an hour before the extract was added.<sup>28</sup>

The extract dissolved in a small amount of dichloromethane was added to the column and the fractions were collected. The polarity of the solvent was gradually increased by adding ethyl acetate to dichloromethane in different ratios ranging from dichloromethane: ethyl acetate (20:1) to (1:1). Fractions containing the same compounds as determined by thin layer chromatography (TLC) were combined and each of the pooled fractions concentrated to dryness under reduced pressure. Fractions

were spotted on a TLC plate and then developed with chloroform: ethyl acetate (6:1). TLC plates were viewed under UV light (254 & 366nm)

### Study of *in-vitro* Antimalarial Activity

The candle jar method of Trager & Jensen,\* (1976) was adopted<sup>30-32</sup>. The parasite used was *Plasmodium falciparum* obtained from outpatient department of Medicine, V.S.S. Medical College, Burla with prior written informed consent from the patients.

The standard drug used was Chloroquine phosphate (IPCA Laboratories Ltd.) and the ethanolic extract of *Nyctanthes arbortristis* was prepared in department laboratory. Other drugs used were Gentamicin injection (Nicholas Piramal India Ltd.), Normal Saline- (Parenteral Drugs India Limited), Heparin (Zydus Cadila Healthcare Ltd.) The chemicals used were Sodium chloride, Sodium hydroxide, Dimethyl sulfoxide (DMSO), Sorbitol, Glycerol, NaHCO<sub>3</sub>, Methanol analytical grade (Sigma-Aldrich). The culture medium was prepared freshly in the laboratory from RPMI-1640 powder (HIMEDIA). JSB (Jaswant Singh–Bhattacharji) Stain (Bio Lab Diagnostics) was prepared in the laboratory. We collected A +ve Blood and inactivated AB+ve Serum from Red Cross Blood Bank, VSSMC, Burla.

The following apparatus and instruments were used in the study. BOD incubator [Tempo Instruments & Equipments (India) Ltd] and Vacuum glass Dessicator (BOROSIL). For plasmodium culture 96 well culture plate (NEST, China), Eppendorf pipette (BD), Sterile micropipette tips (TARSONS) were used. Centrifuge was done with REMI-8C and centrifuge tubes (TARSONS) Liquid nitrogen was procured from (Ankur Agencies). The glass slides were examined with self illuminated light microscope (OLYMPUS, Japan)

### Culture Medium for Cultivation of Malaria Parasite

The standard medium for culture of *Plasmodium falciparum* is RPMI-1640. It was prepared fresh by adding bicarbonates.<sup>33</sup>

### Preparation of erythrocytes for culture

A +ve blood was collected in anticoagulant (heparin). The whole blood was dispensed into aliquots in glass bottles and the required quantity (5 ml.) was put into centrifuge tubes. The centrifuge tubes were spinned @ 1500 rpm for 10 minutes. Then the plasma & buffy coat were removed by Pasteur pipette. Then washing medium was added and put in the centrifuge for spinning @1500 rpm for 10 minutes. The process was repeated 3 times. To it equal amount of Complete RPMI-1640 medium was added and stored at 4<sup>0</sup> C till further use.

### Collection of serum

AB+ ve blood without anticoagulant was kept overnight at 4<sup>0</sup> C. Serum was separated aseptically and kept in aliquots. The serum was inactivated by keeping in water bath 56<sup>0</sup> C for 30 minutes. The inactivated AB +ve serum was stored at -20 to -70<sup>0</sup> C deep freezer for further use.

### Collection of *Plasmodium falciparum* +ve blood samples from the field

Five ml. of blood sample was collected in sterile disposable syringe from patients suffering from *Plasmodium falciparum* malaria, who have not received any antimalarial. In peripheral smear

presence of *Plasmodium falciparum* was confirmed. Blood sample was transferred in heparinised centrifuge tube at 4 °C & transported in coolants to laboratory.

### **Cryopreservation**

The cryoprotectant mixture was made by mixing glycerol 28 ml. with 72 ml. of 4.2% sorbitol in normal saline. The mixture was passed through filter 0.22µm porosity. The infected blood was centrifuged @1500 rpm for 10 minutes and the supernatant was removed. Then equal volume of cryoprotectant (volume of cells) was added. The cryoprotected blood was then distributed in small screw cap vials (cryo tubes). The vials were frozen quickly by immersing in liquid nitrogen.

### **Initiation of culture**

The infected blood was centrifuged and the plasma was removed. The cells were washed with RPMI-1640 washing medium. Then 50% suspension with complete medium was prepared. Appropriate amount of un- infected (washed) cells were added to get initial parasitemia of 0.5-1%. The cells were diluted in complete medium to get 8% parasitemia cell suspension in vials. They were kept in dessicator with a burning candle. Valve was closed when the candle light went out. The dessicator was put in incubator at 37 °C.

### **Stock Solution Preparation:**

First 100 µl RPMI-1640 complete medium was added to in each well. Then 1mg drug was added to 0.9ml. of medium and (up to) 100 µl DMSO (Di-Methyl Sulfoxide) was added so as to make the strength of the drug solution ≈ 1mg/ml. This constituted the stock solution. Then different concentrations of the drug solution were prepared from the solution.

### **Microscopic Test (*in vitro* method)**

A stock solution of 1000 µg/ml was prepared and used. It was serially diluted 10 times in 10 wells and the tenth well remained as control. In each well 10 µl of synchronized (containing only ring stage) parasites was added. Also extra controls were taken in the side wells. Chloroquine was the standard positive control. It was also serially diluted and tenth well remained as control. The cover of the 96 well culture plate was closed and wells were marked over the cover with a permanent marker for chloroquine and NAT. The culture plate was then put in a vacuum glass desiccator. Then a paraffin candle was lit up and waited for till completely burnt. Then the outlet of the stopcock was closed. Thus atmosphere inside the desiccator contained 5% CO<sub>2</sub>, 5% O<sub>2</sub> and 95% N<sub>2</sub>. Then the desiccator was put inside an incubator and the temperature was set at 37 °C and left for 24-36 hours.

After twenty hours the dessicator was taken out of the incubator and the 96 well culture plate was taken out. From the control well 5 µl of blood was pipetted out and thin smear was made. It was stained and fixed with JSB (Jaswant Singh–Bhattacharji) stain and dried under shade. The smear was checked for the growth of ring forms into schizonts. When the maturation to schizont stage was achieved around 70-80% the culture plate was removed and the procedure of staining was repeated. After staining the smears were fixed with methanol. The slides were visualized by a self illuminating microscope under oil immersion. On an average ten fields were looked for i.e. approximately in a count of 5000 erythrocytes. For each slide number of schizonts (parasites containing ≥ 3 nuclei) were counted. Then number of schizonts per 200 parasitized erythrocytes was calculated for each slide. Then doses present in each well were calculated. The data were put in table with an average number

of parasites for individual drug dose. Mean percentage inhibition was calculated by the formula as following

Mean %age inhibition =

$$\frac{\text{Average parasitemia in control} - \text{Average parasitemia in test}}{\text{Average parasitemia in control}} \times 100$$

Then IC<sub>50</sub> was calculated putting log dose on X-axis and the mean percentage inhibition on Y-axis and calculating the dose by deriving the antilog of the log dose which caused 50% inhibition of maturation to schizonts.

### Visual Test

Before performing microscopic test, preliminary bioassay tests were performed. This is a slight modification of the visual *in vitro* test (by Kotecka & Reickmann 1992) to screen extracts for their antimalarial activity. The test relies on the formation of dark pigment precipitates after an alkaline solution is added to synchronous culture of *Plasmodium falciparum*. The pigmentation is produced when maturation of the ring form to schizonts is not prevented by a bioactive product during incubation. This is usually a screening procedure before evaluation by doing a microscopic test.<sup>29</sup>

Microculture was set up and incubated in 5% CO<sub>2</sub> for 48 hours. Then 25 µl of a freshly mixed preparation of 1M sodium hydroxide and 1M sodium chloride was added to the sides of each well of the micro culture plate. The contents of each well turned to a greenish yellow colour and a turbid coagulum was formed. The loose coagulum was due to the denaturation of serum and cell proteins, which takes about 5 minutes until pigment precipitation is formed. The plate was allowed to stand for 20 minutes and tapped again gently to resuspend the precipitate. The wells were examined for the presence of dark precipitates against a light background with a magnifier.

**Statistical Analysis:** All the data were expressed as means and standard deviation (mean ± SD). Unpaired Student's t- test was used for comparison of parasitemia between NAT and CQ.

### RESULTS:

Phytochemical screening of the ethanolic leaf extract of *Nyctanthes arbortristis* revealed the presence of saponins, alkaloids, terpenes, flavonoids and glycosides by column chromatography method.

**Table 1: In vitro antimalarial activity of solvents as determined with the microscopic test (n=20 in each group)**

SOLVENT	Inhibition at 0.2%	Inhibition at 0.1%
Concentrated acetone	20(±5.7)	10(±5.6)
Concentrated dichloromethane	10(±5.0)	0(±3.4)
Concentrated ethanol	0(±5.3)	0(±4.6)
Concentrated petroleum ether	10(±5.4)	0(±5.1)
Dimethyl Sulfoxide	0(±4.8)	0(±2.8)
Ethanol	0(±2.4)	0(±2.1)

**Table 2: Comparison of anti plasmodial activity of ethanolic leaf extract of *Nyctanthes arbortristis* between visual test and microscopic test (n=20 in each group)**

Concentration of Extract in $\mu\text{g/ml}$	Visual test	Microscopic test
10	+	+
5	-	+
2.5	-	+
1.25	-	+

The table compares the antiplasmodial activity by visual test and vitro test of ethanolic leaf extract of NAT. +ve results mean presence of antiplasmodial activity of the NAT extract

**Table 3: Mean percentage inhibition by different concentrations of ethanolic leaf extract of *Nyctanthes arbortristis* (NAT) by in vitro microscopic test (n=20 in each group)**

Sl. No.	Concentration of NAT extract in $\mu\text{g/ml}$	Average number of parasites (Mean $\pm$ SD)	Mean percentage inhibition of maturation to schizonts
1	0	79.5 $\pm$ 1.01	
2	0.2	73.55 $\pm$ 1.14	7.48
3	0.4	68.15 $\pm$ 1.26	14.27
4	0.8	63.35 $\pm$ 1.30	20.31
5	1.6	58.55 $\pm$ 1.23	26.35
6	3.175	53.75 $\pm$ 1.06	32.38
7	6.25	48.60 $\pm$ 1.14	38.36
8	12.5	44.40 $\pm$ 1.27	44.15
9	25	39.50 $\pm$ 1.19	50.31
10	50	34.65 $\pm$ 1.08	56.41

Data are expressed as mean  $\pm$  SD (standard deviation)

**Table 4: Mean percentage inhibition by different concentrations of chloroquine by in vitro microscopic test (n=20 in each group)**

Sl. No.	Concentration of CQ extract in $\mu\text{g/ml}$	Average number of parasites (Mean $\pm$ SD)	Mean percentage inhibition of maturation to schizonts
1	0	79.40 $\pm$ 1.04	
2	0.2	73.10 $\pm$ 1.10	7.93
3	0.4	67.25 $\pm$ 1.27	15.30
4	0.8	62.90 $\pm$ 1.20	20.78
5	1.6	58.15 $\pm$ 0.98	26.72

6	3.125	53.05 ± 1.18	33.18
7	6.25	47.70 ± 1.03	39.92
8	12.5	42.95 ± 1.26	45.90
9	25	38.25 ± 1.25	51.82
10	50	33.85 ± 1.22	57.36

Data are expressed as mean ± SD (standard deviation) (n=20 in each group)

**Table 5: Comparison of schizonticidal activity between NAT & CQ (n=20 in each group)**

Sl. No.	Concentration of drug/ extract in µg/ ml	Schizont Count NAT (Mean ± SD)	Schizont Count CQ (Mean ± SD)	P value
1	0	79.50± 1.01	79.40± 1.04	<b>0.759</b>
2	0.2	73.55± 1.14	73.10± 1.10	<b>0.297</b>
3	0.4	68.15 ± 1.26	67.25 ± 1.27	<b>0.027*</b>
4	0.8	63.35 ± 1.30	62.90 ± 1.20	<b>0.266</b>
5	1.6	58.55 ± 1.23	58.15 ± 1.19	<b>0.244</b>
6	3.175	53.75 ± 1.06	53.05 ± 1.14	<b>0.053</b>
7	6.25	48.60 ± 1.31	47.70 ± 1.05	<b>0.013*</b>
8	12.5	44.40 ± 1.27	42.95 ± 1.26	<b>0.001**</b>
9	25	39.50 ± 1.19	38.25 ± 1.25	<b>0.003*</b>
10	50	34.65 ± 0.97	33.85 ± 1.22	<b>0.035*</b>

Data are expressed as mean ± SD (standard deviation).

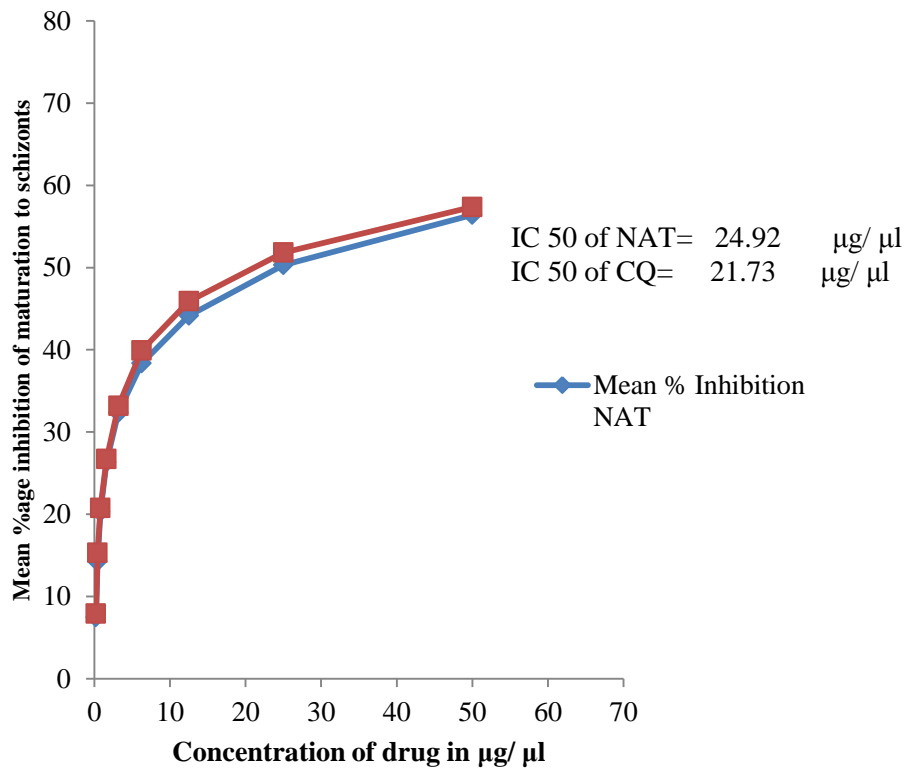
Unpaired t- test was used to analyse the mean count of parasites between NAT and CQ. P value <0.05 was considered significant. P value <0.01 was considered highly significant



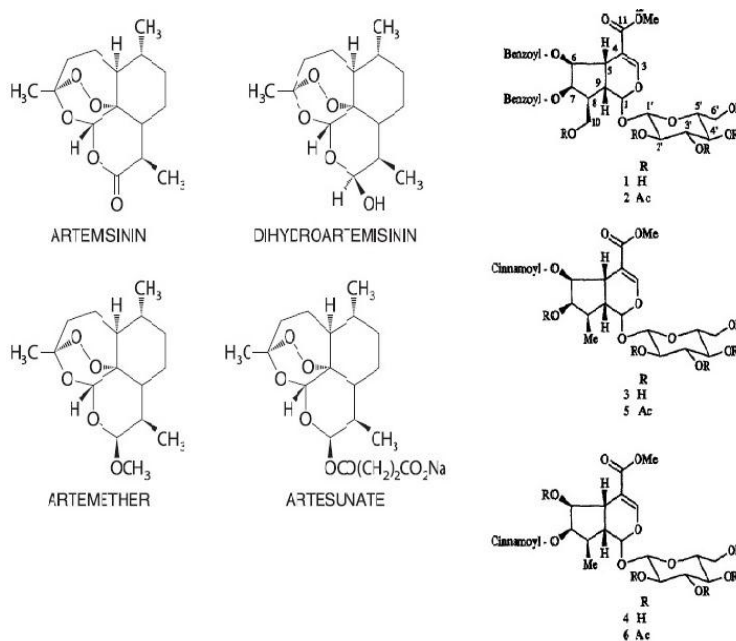
**Figure 1:** The figure shows Leaves with stem of *Nyctanthes arbortristis* (Night Jasmine) plant



IC 50 calculation of NAT & CQ



**Figure 2:** The graph shows inhibitory concentration (in  $\mu\text{g}/\text{ml}$ ) at 50% *Nyctanthes arbortristis* (Night Jasmine) leaf extract (NAT) compared with chloroquine (CQ). The log dose concentration of drug in  $\mu\text{g}/\text{ml}$  is plotted in X-axis and mean percentage of inhibition of maturation to schizonts in Y axis. IC<sub>50</sub> of NAT is 24.92  $\mu\text{g}/\text{ml}$ ., while IC<sub>50</sub> of CQ is 21.73  $\mu\text{g}/\text{ml}$ . The leaf extract (NAT) is comparable to CQ.



**Figure 3:** The figure shows the structural similarity between artemisinin derivatives and iridoid glycosides obtained from *Nyctanthes arbortristis*.

**DISCUSSION:**

*Nyctanthes arbortristis* is widely distributed in Odisha, Eastern State of India. The leaves of this plant have been used traditionally for the treatment of irregular fever in malaria. The present study was conducted to collect evidence that the leaves are effective in the treatment of malaria. We used reverse pharmacology to show laboratory evidence that the ethanolic leaf extract produced inhibition of maturation of *Plasmodium falciparum* schizonts. The IC<sub>50</sub> of NAT (*Nyctanthes arbortristis*) and that of CQ (chloroquine) are comparable. **(Figure: 2)**

Earlier in two clinical studies antimalarial activities have been shown by Karnik et.al (2008) and Godse et.al.(2016).<sup>26-27</sup> Both the studies showed impressive results: that the leaf paste given in patients with malaria showed subsiding of fever and clearing of parasites. There has been a speculation about the mechanism of action of the phytochemical constituents isolated from ethanolic leaf extract (NAT). By column chromatography method the extract yielded alkaloids, flavonoids, glycosides and terpenes. Various studies and reviews indicated that the iridoid glycosides are responsible for the pharmacological activity.<sup>14, 17, 23,24</sup> It has been shown in leishmaniasis that the drug exerts its action by altering the redox potential.<sup>17</sup> It is postulated that like artemisinin, the iridoid glycosides exert their action by producing oxidative stress. **(Figure: 3)**

*Nyctanthes arbortristis* yields many compounds which are useful in many parasitic diseases like leishmaniasis.<sup>17</sup> Even experimentally it has been shown that artemisinin based combination therapy can be done by using iridoid glycosides.<sup>18</sup> The iridoid glycosides also have anti-inflammatory action and it has been reported that they inhibit TNF- $\alpha$ , IL-6 and IL-10.<sup>19,27</sup> This anti-inflammatory action also helps in patients' wellbeing and recovery. The action on platelets also prevents platelet aggregation.<sup>27</sup> In addition the seeds of the plant also yield arbortristides which may open new lines of treatment in cancer therapy.<sup>21</sup>

It is also time tested that some of the plant products like *Picrorrhiza curroa* (Katorohini/ Kutki) which contains iridoid glycosides (Kutkin) has been well documented in Charak Samhita.<sup>12</sup> It has been reviewed that iridoid glycosides may have many medicinal uses. However this plant still has not been patented.<sup>34</sup>

**Conclusion:**

The leaves of night jasmine plant (*Nyctanthes arbortristis*) used in traditional medicine for Visham jwara (malaria) was identified and authenticated by RPRC, Bhubaneswar. By doing reverse pharmacology the chemical constituents were identified. The antiplasmodial activity of the active constituents from ethanolic leaf extract of NAT is comparable to standard drug chloroquine. They were effective in similar doses. Amongst the constituents, iridoid glycosides may be playing a vital role in the treatment of malaria. This needs to be further verified by doing in vivo study and further clinical trials to develop it as an effective antimalarial.

**REFERENCES:**

1. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. *Lancet*. 2018;391(10130):1608-1621.
2. World Malaria Report 2017. Geneva: World Health Organization 2017. Available from <https://www.who.int/malaria/publications/world-malaria-report-2017/en/> Accessed on 15 July 2019.

3. Nkumama IN, O'Meara WP, Osier FHA. Changes in Malaria Epidemiology in Africa and New Challenges for Elimination. *Trends Parasitol.* 2017;33(2):128-140.
4. Brunton, Laurence L, and Keith L Parker. "Chemotherapy of Protozoal Infection: Malaria." *Goodman and Gilman' Manual of Pharmacology & Therapeutics*, 13th ed.,Mc Graw-Hill Education. 2018, p. 970.
5. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. *Lancet.* 2014;383(9918):723-735.
6. Bettencourt P. Current Challenges in the Identification of Pre-Erythrocytic Malaria Vaccine Candidate Antigens. *Front Immunol.* 2020;11:190.
7. Zheng J, Pan H, Gu Y, et al. Prospects for Malaria Vaccines: Pre-Erythrocytic Stages, Blood Stages, and Transmission-Blocking Stages. *Biomed Res Int.* 2019;2019: 9751471.
8. Frimpong A, Kusi KA, Ofori MF, Ndifon W. Novel Strategies for Malaria Vaccine Design. *Front Immunol.* 2018;9:2769
9. Brunton, Laurence L, Lazo John S and Keith L Parker. "Chemotherapy of Protozoal Infection: Malaria." *Goodman and Gilman' Manual of Pharmacology & Therapeutics*, 11th ed., The McGraw-Hill Companies Inc.2006, p. 1035
10. White NJ. Qinghaosu (artemisinin): the price of success. *Science.* 2008;320(5874):330-334..
11. Tu Y. The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nat Med.* 2011;17(10):1217-1220
12. Sharma PV- Editor. Chakradutta Chikitsastanam Caraka Samhita (Text with English Translation). Vol. II. 4<sup>th</sup> Edn. Chaukhamba Orientalia, Varanasi: 1998. p. 58-59,72
13. Ambasta SP. *Nyctanthes* Linn. *Oleaceae*. The Useful Plants of India. Publications & Information Directorate CSIR, New Delhi; 1986 p.400-401
14. Parekh S, Soni A. *Nyctanthes arbor-tristis*: Comprehensive review on its pharmacological, antioxidant, and anticancer activities. *J Appl Biol Biotech.* 2020;8(01):95–104.
15. Jain PK, Pandey A. The wonder of Ayurvedic medicine-*Nyctanthes arbortristis*. *Int J Herb Med.* 2016; 4(4):9-17.
16. Agrawal J, Pal A. *Nyctanthes arbor-tristis* Linn--a critical ethnopharmacological review. *J Ethnopharmacol.* 2013;146(3):645-658.
17. Shukla AK, Patra S, Dubey VK. Iridoid glucosides from *Nyctanthes arbortristis* result in increased reactive oxygen species and cellular redox homeostasis imbalance in Leishmania parasite. *Eur J Med Chem.* 2012;54:49-58
18. Singh AK, Pal A, Dutt HK. Antimalarial efficacy of *Nyctanthes arbortristis* and its effect on combination with Artesunate in *Plasmodium berghei* K173 induced mice model. *Int J Clin Pharmacol Res* 2019;8(1):01-09
19. Agrawal J, Shanker K, Chanda D, Pal A. *Nyctanthes arbor-tristis* positively affects immunopathology of malaria-infected mice prolonging its survival. *Parasitol Res.* 2013;112(7):2601-2609
20. Saxena RS, Gupta B, Saxena KK, Singh RC, Prasad DN. Study of anti-inflammatory activity in the leaves of *Nyctanthes arbor-tristis* Linn.--an Indian medicinal plant. *J Ethnopharmacol.* 1984;11(3):319-330

21. Bharshiv CK, Garg SK, Bhatia AK. Immunomodulatory activity of aqueous extract of *Nyctanthes arbor-tristis* flowers with particular reference to splenocytes proliferation and cytokines induction. *Indian J Pharmacol.* 2016;48(4):412-417
22. Mishra RK, Mishra V, Pandey A, et al. Exploration of anti-Malassezia potential of *Nyctanthes arbor-tristis* L. and their application to combat the infection caused by *M. s1* a novel allergen. *BMC Complement Altern Med.* 2016;16:114
23. Khan ZK, Manglam A, Shukla PK, Puri A, Saxena RP, Tandon JS. Immunomodulatory effect of plant and iridoid glycosides from *Nyctanthes arbor-tristis* Linn. against systemic candidiasis in mice. *Pharm Biol* 1995;33(4):297-304.
24. Stuppner H, Muller EP, Mathuram PV, Kundu AB. Iridoid Glycosides from *Nyctanthes arbor-tristis*. *Phytochemistry* 1993; 32 (2): 375-378.
25. National Framework for Malaria Elimination in India (2016-2030). Ministry of Health & Family Welfare, Government of India. Available from: <http://www.indiaenvironmentportal.org.in/files/file/National-framework-for-malaria-elimination-in-India-2016-2030.pdf>, accessed on July 15, 2019.
26. Karnik SR, Tathed PS, Antarkar DS, Godse CS, Vaidya RA, Vaidya AB. Antimalarial activity and clinical safety of traditionally used *Nyctanthes arbor-tristis* Linn. *Indian J Tradit Knowl.* 2008;7(2):330–334
27. Godse CS, Tathed PS, Talwalkar SS, et al. Antiparasitic and disease-modifying activity of *Nyctanthes arbor-tristis* Linn. in malaria: An exploratory clinical study. *J Ayurveda Integr Med.* 2016;7(4):238-248.
28. Salituro, Gino M. (et al.) Isolation by Low-Pressure Column Chromatography. Cannell, Richard J. P. Editors *Natural Products Isolation* 4<sup>th</sup> Ed., Humana Press, Totowa, New Jersey : 1998 p.111-140
29. Kotecka B, Rieckmann KH. An inexpensive and simple method for screening potential antimalarial drugs. *Trop Med Parasitol.* 1992; 43(1):9-12.
30. Jensen JB , Trager W *Plasmodium falciparum* in Culture: Use of Outdated Erythrocytes and Description of the Candle Jar Method *J. Parasitol;* 1977: 63: 5: 883-886
31. Trager W, Jensen JB Human malaria parasites in continuous culture. *Science;* 1976: 193: 673–675.
32. Trager W, Jensen JB. Cultivation of erythrocytic stages. *Bull World Health Organ.* 1977; 55(2-3):363-365.
33. Schuster FL. Cultivation of *Plasmodium* spp. *Clin Microbiol Rev.:*2002; 15:3: 355–364.
34. Hussain H.; Green I.R.; Saleem M.; Raza M.L.; Nazir M. Therapeutic Potential of Iridoid Derivatives: Patent Review. *Inventions* 2019, 4(2) 29.

**ACKNOWLEDGEMENT:** We are thankful to

1. Dr. Pratap Panda, PhD., Scientist at Regional Plant Research Centre, Bhubaneswar for identification and authentication of the Night Jasmine plant (*Nyctanthes arbortristis*) and preserving the sample in herbarium.
2. Staff of National Institute of Malaria Research (NIMR), Delhi for their help and support.