

(Final progress report of the project from November 2012 to 2015)

Project title: *Screening and Isolation of Bioactive Compounds from Medicinal plants traditionally used against malaria in Arunachal Pradesh.*

Sanction No. and date: DRL/1047/TC Date: 5.07.2012 (DRLT-P1-2012/Task-56)

1. Introduction

Malaria, a mosquito borne infectious disease of humans and other animals is caused by parasitic protozoan of the genus *Plasmodium*. It is estimated to be responsible for ~250 million clinical episodes and nearly 1 million deaths annually worldwide and is most prevalent particularly in poorer tropical and subtropical regions. There are many species of *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium bubalis*, *Plasmodium juxtannucleare*, *Plasmodium circumflexum*, *Plasmodium elongatum*, *Plasmodium coatneyi*, *Plasmodium cynomolgi* and *Plasmodium ovale*. Among all the species *P. falciparum* and *P. vivax* is mainly responsible for causing malaria in human. The former causes more than one million deaths each year, mainly young children in synergy with other infections and illnesses. In India, malaria is predominantly a rural disease where agriculture farming especially rice through irrigation method is a common practice by the tribal people, may provide suitable microhabitats for mosquito breeding. Irrigated rice farming, rearing of domestic animals, fishpond, and lack of proper drainage system constitute an additional factor contributing the mosquito breeding and malaria infections¹.

Historically, majority of anti-malarial drugs have been derived from medicinal plants or from structures modeled on plant derived compounds². These include anti-malarial properties of *Cinchona* bark, known for more than 300 years and several semisynthetic derivatives of artemisinin, the active ingredient of the Chinese herb 'Qinghao' (*Artemisia annua*, which was used traditionally for treating fevers) which have been used increasingly over the past two

decades. Within the context of traditional practice, malaria (or malaria symptoms) is commonly treated by decoctions or infusions from bitter plants³⁻⁸. Several classes of bioactive plant metabolites are known to be responsible for anti-plasmodial activity, the most potent and important has been observed in alkaloids, quassinoids, sesquiterpene lactones, coumarins, triterpenoids, limonoids, and quinines⁹.

Malaria is endemic in most north-eastern region of India with preponderance of *Plasmodium falciparum* infection (60–90%) being the main characteristic of the disease pattern in the region. Difficult terrain, hilly forests, inadequate infrastructure coupled with the development of chloroquine resistance in *P. falciparum* in Assam further aggravates the situation. In Assam, most cases of malaria were caused by *P. falciparum* infections while the remaining was by *P. vivax* infections as per the data collected from the Army Hospital units based in Assam. The factors such as drug resistance, low efficacy, safety issues, poor compliance, and high cost, especially in poorer nations has become increasingly common in the recent years. Thus, resistance to currently used anti-malarial drugs such as chloroquine and artemisinin necessitates continuing efforts to develop new drugs, ideally with novel modes of action against both the liver and blood stages of the parasite. Therefore, the present aim of the project is to isolate and characterize the bioactive compounds from selected traditional anti-malarial plants and understanding the mechanism of action would help us to develop strategies to improve anti-malarial therapy and effective management of malaria in the north-eastern region of India including Assam.

However, till date complete characterization of compounds from medicinal plants traditionally used against malaria has not been thoroughly investigated in India. Therefore, further isolation, identification, purification, characterization and understanding the pharmacological efficacy (anti-malarial activity) of plant-derived compounds is needed for effective management of malaria. Purification and characterization of these compounds from plants can be successfully done with the help of various chromatographic, GC-MS and NMR techniques. The pharmacological effects of these compounds can be evaluated using experimental animal models. Thus, this would prove to be a potential therapeutic application in biotechnology and warfare that would offer effective and alternative affordable management of emergence of multi-drug resistant malaria.

2. Objectives

2.1. Field survey, documentation, sample collection and generation of database of traditional anti-malarial plants from Arunachal Pradesh.

2.2. Preliminary screening for in vitro anti-plasmodial and anti-larvicidal activity of crude extracts from selected plants.

2.3. Isolation, characterization and anti-plasmodial activity of bioactive compounds in animal model.

3. Materials and methods

3.1. Survey and plant sample collection

On the basis of ethno-pharmacological knowledge plant samples were collected from different part of Arunachal Pradesh and Assam. It revealed that folk medicine is still widely practiced by the population in the eastern Himalayan province of Arunachal Pradesh, and the use of medicinal plants continues to be a viable healthy alternative for the large underprivileged section of the ethnic population. It also showed that herbal decoctions (single or multiple herbal decoctions) are widely used as prophylactic agent against malaria by the rural people of the state.

3.2. Preparation of plant extract

Collected samples were air dried at room temperature and grounded to powder using mortar and pestle or grinder. Then 20 gram of powdered plant material was dissolved in 200 ml of water, methanol, butanol, and n-hexane and kept for 48 hrs at room temperature. After 48 hrs plant material dissolved in water was heated at 40⁰ C and filtered. The plant material dissolved in methanol, butanol and n-hexane were filtered after 48 hrs and allowed to evaporate completely at room temperature. Extract was collected and dissolved in 1% DMSO to make the conc. 10mg/ml (stock) and stored at -20°C for various assays.

3.3. Larvicidal activity

A laboratory colony of *Aedes albopictus* was used for the larvicidal activity of fractions collected at the two stages. Twenty late third instar larvae and early fourth instar larvae were kept in 500 ml glass beaker containing aqueous suspension of fraction at dilution from 5ppm to 500ppm. Three replicates were set up for each dilution. The negative control was exposed to water. Larval mortality was assessed after 24 hrs, 48 hrs of exposure by probing the larvae with needle. A dose-dependent mortality and the lethal concentration of fraction needed to kill 50% (LC50) of larvae have been determined.

3.4. Anti-microbial activity

The effect of plant extract on different bacterial strains was determined by the agar-well diffusion method. Bacterial cultures of *S. aureus* (MTCC 3160), *B. subtilis* (MTCC 121), *K. pneumoniae* (MTCC 618), *M. smegmatis* (MTCC) were grown overnight in LB medium. The 100 µL of the culture was spread uniformly on MHA plates. 100µl of 10mg/ml plant extract [methanol extract (ME)] were loaded into the wells formed on plates containing lawn of different bacteria. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of zone of inhibition (mm) around the well after 24 hours (NCCLS,1993). Antibiotic Ampicillin was used as positive control.

3.5. Purification of plant extracts

Preliminary purification of the crude plant extracts for the presence of different classes of natural products was done by performing Thin Layer Chromatography (TLC) (Harborne, 1973). The plant extracts were subjected to TLC to separate the active compounds present. The plates were prepared using a slurry of silica gel G in distilled water. Silica gel G (1g) was added to 3ml of distilled water and a thick slurry was made. All solid particles were blended well, and the uniform silica gel slurry was applied onto the TLC plate at a thickness of 1mm to 1.2 mm. The plate was allowed to dry at room temperature. The dried plate was placed in the oven at 100°C for 30 minutes to activate the silica gel. The plate was taken from the oven and kept at room temperature for 15 minutes.

Using a micro-capillary tube, a small drop of methanolic plant extract was placed on the TLC plate, 1cm above the bottom. This spot was allowed to dry, and the TLC plate was placed into the TLC chamber which was saturated with the solvent mixture carefully to have uniform solvent level. When the solvent reached 2 cm below the top, the plates were taken out of the chamber and detected with the respective spraying reagents. The chromatogram was developed with different solvents namely, Ethyl Acetate: Hexane, Acetone: Diethylether, Acetone: Chloroform, Acetone: Ethyl Acetate in different ratio particularly 10, 8:2, 6:4, 4:6, 2:8.

The R_f values of the TLC spots were calculated by the formula,

$$R_f = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

4. Results

4.1. Sample Collection

Based on the ethno-pharmacological knowledge 15 plant samples were collected from different parts of Arunachal Pradesh and Assam known to treat malaria. From the survey, we could find out that mostly the roots, bark, stem, leaves were used traditionally to treat malaria by the local people (Table 1).

Table1: Traditional information on anti-malarial plants used by indigenous tribe of Arunachal Pradesh

Scientific name	Family name	Local name	Habit	Parts used	Herbal formulation
<i>Thalictrum foliolosum</i> DC.	<i>Ranunculaceae</i>	Yangchura	Herb	Roots	Dried root powder mixed with <i>Thymus linearis</i> in equal proportion is taken regularly.
<i>Aristolocia saccata</i> Wall.	<i>Aristolochiaceae</i>	Nagbol	Shrub	Roots	Tuber extracts are used.
<i>Solanum khasianum</i> Cl.	<i>Solanaceae</i>	Thityake[N]	Shrub	Roots	Decoction of root for 6 days.
<i>Cinchona officinalis</i>	<i>Rubiaceae</i>		Tree	Bark	Powdered bark is taken with warm water for 7 days.
<i>Coptis teeta</i> Wall.	<i>Ranunculaceae</i>	Mishmi teeta [AT]	Herb	Roots	1 glass of boiled root is given for 3 days.
<i>Zanthoxylum hamiltonianum</i> Wall.	<i>Rutaceae</i>	Ombe [AG]	Tree	Roots and Bark	Decoction of root and bark is taken for 3 days.
<i>Begonia ruxburghii</i> (Miq) DC	<i>Begoniaceae</i>	Baya [N]	Herb	Roots , Leaves and Petiole	Root, Leaves and petiole are eaten raw for 5 days.
<i>Dendrocide sinuata</i> (Bl.) Chew	<i>Urticaceae</i>	Podret [N]	Shrub	Leaves	Leaves are mixed with leaves of <i>Stephania glabra</i> (2:1) and boiled

					in water. 1-2 teaspoonful of solution is administered for 6 days.
<i>Dioscorea belophylla</i> Voigt ex Haines	<i>Dioscoreaceae</i>	Yazeng pep [N]	Climber	Tubers	Tubers are crushed and mixed with hot water about ½ tea spoon given twice day for 3 days.
<i>Indofevillea khasiana</i> Chatterjee.	<i>Cucurbitaceae</i>	Yazang-pipe [N]	Climber	Roots and Stem	About 5 gm powdered root and stem is taken with warm water, twice a day for 5 days.
<i>Maesa macrophylla</i> Wall.	<i>Myrsinaceae</i>	Tak Sangne [N]	Shrub	Fruits	5-6 berries are eaten fresh thrice a day for 5 days.
<i>Melothria heterophylla</i> (Lour) Cogn.	<i>Cucurbitaceae</i>	Yazang pipe [N]	Herbs	Tuber	5-10 gm of pounded tubers is taken with a glass of warm water for 3 days.
<i>Solanum myriacanthum</i> Jacq.	<i>Solanaceae</i>	Thit byako [N]	Shrub	Roots	1-3 teaspoonfuls of decoction of roots are taken twice a day for 7 days.
<i>Solanum turvum</i> Sw.	<i>Solanaceae</i>	Bako (N)	Shrub	Roots	Decoction of root.
<i>Swertia chirata</i> (Wall.) C. B. Clarke	<i>Solanaceae</i>	-	Herb	Aerial parts	A dried herb is soaked in water for 12 hours (Evening). A glass of solution is taken twice a day for 6 days.

Note: N- Nishi; AT-All tribe; AG- Adi Galo

4.2. Larvicidal Activity

The larvae of *Aedes albopictus* did not show any significant mortality in the presence of plant extract *Thalictrum foliolosum*, and *Aristolochia saccata*. The plant extract used was mostly in the form of crude extract.

4.3. Anti-microbial Activity

The plant extract was allowed to diffuse out into the medium and interact with the bacterial strains. Growth inhibition was determined by measuring zone of inhibition after 24 h. The resulting zone of inhibition formed were uniform circular zones around the well. Preliminary investigation with crude extracts of a few plants (*Thalictrum foliolosum*, *Aristolochia saccata*) showed antibacterial activity against gram-positive bacteria as well as gram-negative bacteria (Fig.1).

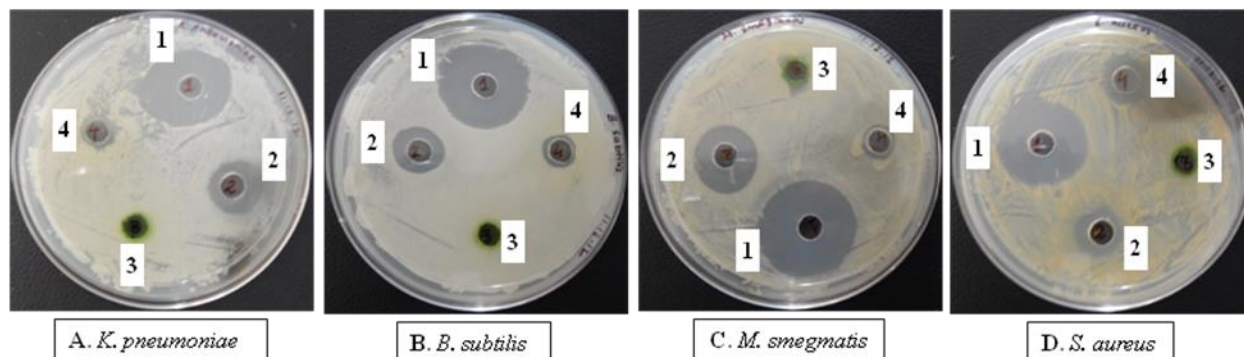


Figure 1. Anti-bacterial activity of methanolic extract (ME) of *Thalictrum foliolosum* , *Aristolochia saccata* by agar well diffusion method. 100 μ l of 10mg/ml ME were loaded into the wells formed on plates containing lawn of *K. pneumoniae* (A), *B. subtilis* (B), *M. smegmatis* (C), *S. aureus* (D). (1)Antibiotic, (2) *Aristolochia saccata* plant extract, (ME), (3) Jor paat plant extract (ME), (4) *Thalictrum foliolosum* plant extract (ME). Antibiotic Ampicillin was used as control for bacterial strain.

4.4. Partial purification by TLC

The Rf values for different spots for different extracts were determined and results have been tabulated in Table 2 along with the photographs in Fig.2. From the above work carried out of the methanolic plant extract indicated the presence of Alkaloids and Phytosterols. This extract was further analyzed by TLC and spots were identified in the solvent systems with the following ratio's i.e. Ethyl Acetate: Hexane, Acetone: Diethylether, Acetone: Chloroform, Acetone: Ethyl Acetate in different ratios 10, 8:2, 6:4, 4:6, 2:8, 10.

In this regard, we can conclude that as compounds are present in the extracts of this plant, further purification has to be carried out to explore *Thalictrum foliolosum* for its further research activity. The main objective of the proposal could not be achieved due to lack of culture facility for malaria parasite at Entomology Division, DRL, Tezpur.

Table 2. TLC protocol and Rf value

COMPOUNDS	MOBILE PHASE	Ratio	Rf value
Phytosterols	Ethylacetate: Hexane	10 Ethylacetate	0.533
		8:2	0
		6:4	0.306
		4:6	0.241
		2:8	0
		10 Hexane	0
	Acetone: Diethylether	10 Acetone	0.75
		8:2	0.721
		6:4	0.667
		4:6	0
		2:8	0
		10 Diethylether	0
Alkaloids	Acetone: Chloroform	10 Acetone	0.769
		8:2	0.667
		6:4	0.892
		4:6	0.8
		2:8	0.796
		10 Chloroform	0.488
Alkaloids	Acetone: Ethylacetate	10 Acetone	0.733
		8:2	0.613,0.8
		6:4	0.562,0.75
		4:6	0.625
		2:8	0.563
		10 Ethylacetate	0.486

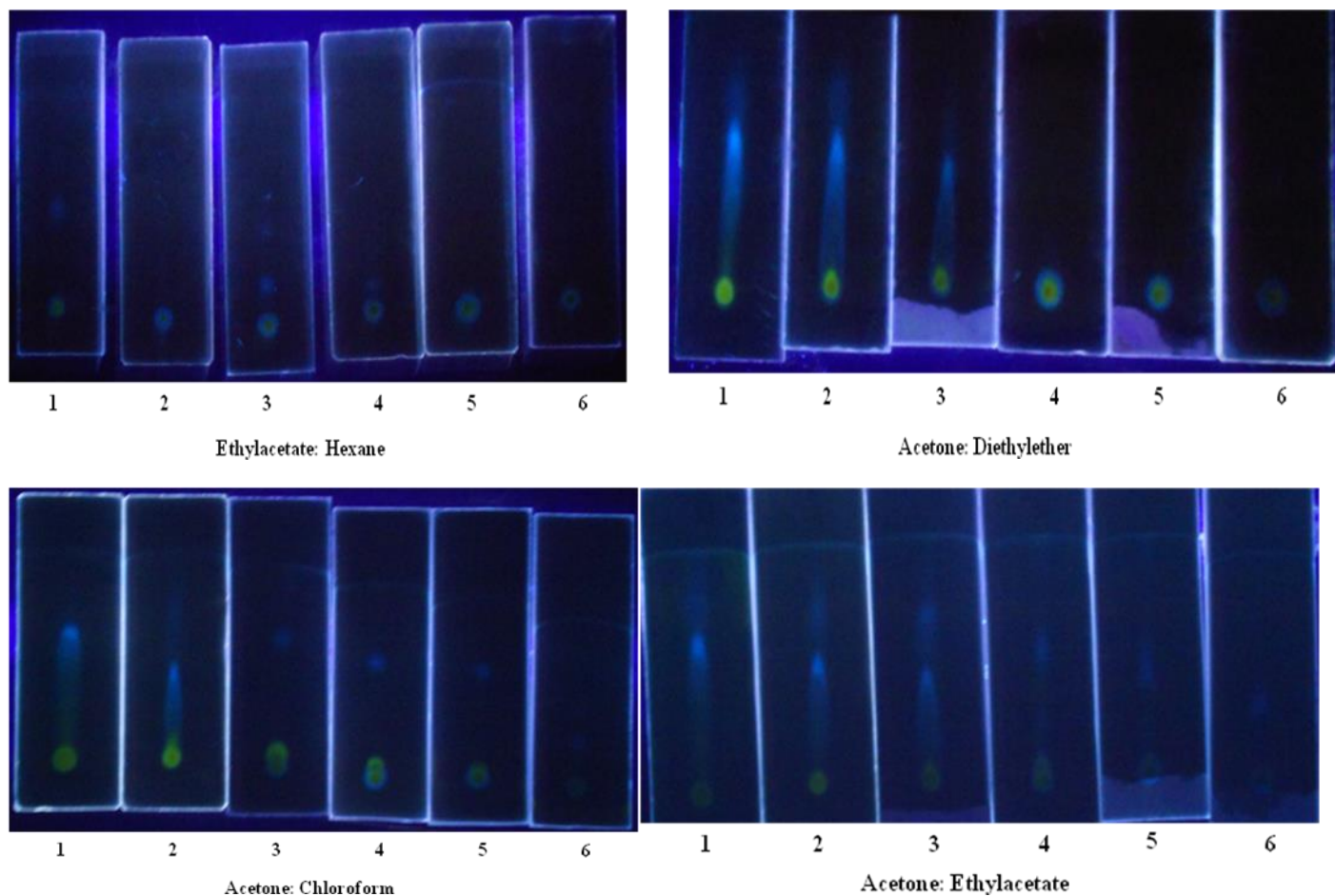


Figure 2. Images of TLC plates showing spots depicting the presence of alkaloids and phytosterols when run in different solvent solution.

Study on midgut bacterial fauna in *Aedes* mosquitoes: vector of Dengue and Chikungunya disease

We have also carried out the midgut bacterial association in *Aedes* mosquitoes: vector of Dengue and Chikungunya disease under this project at DRL, Tezpur.

Vectors play an important role in transmission of various diseases such as malaria, dengue fever, Chikunguniya, Leshmaniasis, Encephalitis, Rickettsial diseases, West Nile virus, etc. Dengue is the most rapidly spreading mosquito-borne viral disease throughout tropical and sub-tropical regions of the world, with a 30-fold increase in global incidence over the past 50 years. The four serotypes of Dengue virus (DENV) of family *Flaviviridaie* are transmitted by the *Aedes*

mosquito vectors that is the causative agent of dengue fever. No effective antiviral agents like drugs and vaccines available to treat dengue infection. Thus, there is a need to develop a cheap, safe, and eco-friendly, tool for the control of dengue and without the fear from evolution of resistance. Many studies have been reported about the rich community of midgut microbiota of mosquitoes that play important role in interaction with invading pathogens and its involvement in defense mechanism. Very limited studies are available on *Aedes* mosquitoes with respect to gut microflora identification and its interaction with the disease transmitting agent. The present study was carried out to understand the midgut bacterial population of *Aedes (Stegomyia) albopictus* collected from different region of North-east India. Using of midgut bacteria is the new and more effective strategies for mosquito control and protection against the transmission of disease, but little is known about the bacteria flora within the mosquito's midgut and key question regarding composition, stability and acquisition remain largely unanswered. Thus, there is urgent need to initiate a study to identify symbiotic bacteria inhabiting the midgut of mosquitoes, with the purpose of selecting symbionts potentially useful for the development of para-transgenic strategies for disease control. The proposed work is expected to help in appropriate symbiotic bacteria selection for the development of defense mechanism against invading pathogens and in disease transmission prevention.

Objectives:

- To study midgut microbial community structure of *Aedes* mosquito from different regions.
- Isolation of culture dependent bacteria.
- Morphological and biochemical characterization of bacterial isolates.
- Molecular characterization of cultured independent bacteria of mosquito's midgut form different field station.
- Molecular identification and phylogenetic analysis of isolates based on 16S rRNA gene sequence using different bioinformatics tools.
- To study geographical variation and diversity indexes in bacterial community structure form different field station.

- **Collection of *Aedes* mosquito from different regions of North-east India:**

Table 3- Sample collection sites:

S. No.	Place of Sample Collection	Mosquito Species (Female)
1.	*Tezpur, Assam	<i>Aedes albopictus</i>
2.	Demoruguri, Nagaon, Assam	<i>Aedes albopictus</i>
3.	Mesamari Army Cantt. (near air base), Assam	<i>Aedes albopictus</i>
4.	Bhalupong City, Arunachal Pradesh	<i>Aedes albopictus</i>
5.	Bhalupong City, Arunachal Pradesh	<i>Aedes aegypti</i>

***Tezpur 4 group of sample collected:** 1-Blood fed female mosquitoes; 2-unblood fed female mosquitoes; 3- Male mosquitoes; 4- Larvae

- **Mosquitoes dissection for midgut bacterial flora isolation and morphology study:**
 - Mosquito's dissection (Collected from different field stations) and midgut separation for aerobic bacteria isolation in sterile condition.
 - Culturing and purification of isolated aerobic bacteria.
 - Sub culturing and purified bacteria stored in 40% glycerol stock.
 - Morphology characteristic (size, shape, colour, opacity, margin, elevation, motility, number of colony) were studied.
 - Pool preparation of 100 midguts for study of uncultured bacteria.
- **Isolation of DNA from bacterial flora and PCR amplification:**
 - Isolation of DNA from all bacterial isolates recovered from different field stations.
 - PCR amplification of 16S rRNA gene using 16S rRNA universal primers.
 - Purification and sequencing of PCR amplicons.
- **Sequence analysis and data compilation:**
 - Sequences of 17 bacterial isolates have been obtained and it is alignment, and analysis the BLASTn of sequence.
 - Similar sequence was downloaded from GenBank for phylogenetic analysis.

- Phylogenetic analysis of obtained sequence of 16 samples has been done.

Table 4- Identified bacterial isolates of obtained 17 sequences:

S.No.	Isolates	Species Name	Phylum	Class	Order	Family
1	B1a1	<i>Klebsiella oxytoca</i>	Proteobacteria	Gamma Proteobacteria	Enterobacteriales	Enterobacteriaceae
2	B13a1	<i>Enterobacter asburiae</i>	Proteobacteria	Gamma Proteobacteria	Enterobacteriales	Enterobacteriaceae
3	S17b1	<i>Enterobacter asburiae</i>	Proteobacteria	Gamma Proteobacteria	Enterobacteriales	Enterobacteriaceae
4	S21b2	<i>Enterobacter asburiae</i>	Proteobacteria	Gamma Proteobacteria	Enterobacteriales	Enterobacteriaceae
5	BPA10-4a1	<i>Enterobacter hormaechei</i>	Proteobacteria	Gamma Proteobacteria	Enterobacteriales	Enterobacteriaceae
6	S16a2	<i>Leclercia adecarboxylata</i>	Proteobacteria	Gamma Proteobacteria	Enterobacteriales	Enterobacteriaceae
7	BPB10-4C1	<i>Pseudomonas beteli</i>	Proteobacteria	Gamma Proteobacteria	Pseudomonadales	Pseudomonadaceae
8	S4b1	<i>Flavimonas oryzihabitans</i>	Proteobacteria	Gamma Proteobacteria	Pseudomonadales	Pseudomonadaceae
9	S5a1	<i>Pseudomonas mosselii</i>	Proteobacteria	Gamma Proteobacteria	Pseudomonadales	Pseudomonadaceae
10	S19b2	<i>Aeromonas veronii</i>	Proteobacteria	Gamma Proteobacteria	Aeromonadales	Aeromonadaceae
11	S17d2	<i>Staphylococcus hominis</i>	Firmicutes	Bacilli	Bacillales	Staphylococcaceae
12	S21a1	<i>Elizabethkingia anophelis</i>	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae
13	MS1	<i>Bacillus flexus</i>	Firmicutes	Bacilli	Bacillales	Bacillaceae
14	B11_1	<i>Micrococcus yunnanensis</i>	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
15	B11_2	<i>Clostridium sporogenes</i>	Firmicutes	Clostridia	Clostridiales	Clostridiaceae
16	B11_3	<i>Bacillus aryabhatai</i>	Firmicutes	Bacilli	Bacillales	Bacillaceae
17	B11_4	<i>Bacillus altitudinis</i>	Firmicutes	Bacilli	Bacillales	Bacillaceae

5. Research papers published in journals and conferences (This part of work was published by DRL, Tezpur)

5.1. Yadav KK, Datta S, Naglot A, Bora A, Hmuaka V, Bhagyawant S, et al. (2016). Diversity of Cultivable Midgut Microbiota at Different Stages of the Asian Tiger Mosquito, *Aedes albopictus* from Tezpur, India. PLoS ONE 11(12): e0167409.

6. Future objectives

1. *In vitro* testing of the anti-plasmodial activity of the selected plants

- a. Schizont maturation inhibition assay
- b. *Plasmodium falciparum* lactate dehydrogenase inhibition assay (PfLDH),
- c. Plasmeprin II inhibition assay

2. Pharmacological efficacy of partially isolated compounds using various *in vitro* and *in vivo* experimental models

7. References

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Appendix-B

Utilization Certificate
(For the financial year ending 31st March 2015)

1. Title of the Project/Scheme: "Screening and isolation of bioactive compounds from medicinal plants traditionally used against malaria in Arunachal Pradesh".
2. Name of the Organization: **Dept. of Mol. Bio & Biotech, Tezpur University**
3. Principal Investigator: **Dr. Nima D. Namsa**
4. Sanction order No. & date of sanctioning the project: **DRL/1047/TC Date: 5.07.2012**
5. Amount brought forward from the previous financial year in which the authority to carry forward the said amount was given : **Rs. (-) 14,590**
6. Amount received from DRDO during the financial year (2014-15) : **Rs. 4,32,000**
7. Total amount that was available for expenditure during the financial year : **Rs. 4,17,410**
8. Actual expenditure (excluding commitments) incurred during the financial year : **Rs. 3,82,456**
9. Balance amount available at the end of the financial year : **Rs. 5531**

Certified that the amount of Rs. 3,82,456 mentioned against col. 8 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the remaining unspent balance of Rs. 5531 is refunded via demand draft No. 048545 dated 13/07/2017.

Certified that I am myself satisfied that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Namsa
28/07/17
(Project Investigator)
Assistant Professor
Dept. of MBBT
Tezpur University

S. Namsa
28/07/17
(Finance Officer)
Finance Officer
Tezpur University

B
(Head of the Institute)
Registrar
Tezpur University

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS

Surajit Chakraborty
09.08.2017
CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

5. Statement of Expenditure

Sl No.	Head	First Year Grant received (Rs.) (I)	Second Year Grant received (Rs.) (II)	Total amount received (Rs) (III)	Interest earned, if any, on the grants received	Amount Utilized (Rs.)			Total expenditure incurred (Rs) VII (IV+V+VI+VII)	Unspent balance (Rs) VIII (III-VII=VIII)
						2012-13 (IV)	2013-14 (V)	2014-15 (VI)		
1	Staff Salary	2,88,000	2,88,000	5,76,000		1,20,000	1,92,000	2,38,065	5,50,065	25,935
2	Equipment	Nil	Nil							
3	Consumable	60,000	24,000	84,000		70,270		13,030	83,300	700
4a	Misc. Expt (Contingency)	5,000	5,000	10,000		5,003		5,000	10,003	-3
4b	Misc. Expt (50% Overhead)	90,000	90,000	1,80,000		35,017	45,000	90,000	1,70,017	9,983
5	Travel	25,000	25,000	50,000		Nil	15,300	36,361	51,661	-1,661
6	Refund of unspent balance									34,954 (Unspent balance of Rs. 34954 was refunded to Director, DRL Tezpur via demand draft No. 137566 dated 23/06/2016.
7	Interest earned for the period 24/09/2012 to 31/03/2015.				5531				00	5531
Total		4,68,000	4,32,000	9,00,000		2,30,290	2,52,300	3,82,456	8,65,046	5,531

Surajit
24/07/17
(Project Investigator)
Assistant Professor
Dept. of MBBT
Tezpur University

B
Surajit
24/07/17
(Finance Officer)
Finance Officer
Tezpur University

For SURAJIT CHAKRABORTY & CO
CHARTERED ACCOUNTANTS

Surajit
24/07/17
CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No. 305054

R
(Head of the Institute)
Registrar
Tezpur University