Current Trends in **Pharmaceutical Research** Vol. 6 Issue 1, 2019
ISSN:2319-4820

www.dibru.ac.in/ctpr

Research article

PRELIMINARY VALIDATION OF ETHNOMEDICINAL PLANTS USED IN THE PREPARATION OF ANTIMALARIAL HERBAL REMEDIES BY *IN VITRO* SCREENING METHOD

Neelutpal Gogoi* and Dipak Chetia

Department of Pharmaceutical Sciences, School of Science and Engineering
Dibrugarh University, Dibrugarh-786004, Assam, India

Abstract

Background*: Malaria is one of the deadliest infectious diseases, which still gain attention from the drug discovery scientist due to development of resistant towards the available drugs.* ***Objective:*** *In the present study, three ethnomedicinal plants have been evaluated for in vitro antimalarial activity to validate their use.* ***Materials & Methods:*** *Processed plant materials were extracted with hydroalcoholic solvent (1:1) by cold maceration techniques for 72 hours. The extracts were collected by evaporating the solvents using rotary vacuum evaporator followed by freeze drying by lyophilisation. The antimalarial activities of the extracts were evaluated against chloroquine sensitive (3D7) and resistant (RKL-9) strain of Plasmodium falciparum by observing inhibition of schizont maturation using Giemsa straining method. The* IC_{50} *value of the extract were determined by serial drug dilution techniques and results were interpreted using statistical software NonLin v1.1.* ***Results and discussion****: From the preliminary antimalarial screening, it was observed that the extracts of all the three plants have shown high (<10*

*Corresponding author's E-mail: neelutpalg@gmail.com

µg/ml) activity against both sensitive (3D7) and resistant (RKL-9) strains of Plasmodium falciparum, where the extract of Artemisia nilagirica (Clarke) Pamp. has shown very good activity in comparison to other extracts. Although the results were not comparable with the standard drug chloroquine, but at extract level the results were quite promising to go for bioactivity guided investigation of potent antimalarial compounds. ***Conclusion****: The study has validated the traditional use of the plant materials as antimalarial herbal remedy and guided towards investigation of new compounds from the active plant materials.*

Key words: *Traditional practice, Medicinal plant, Malaria, North-east India*

Introduction

Malaria is one of the lethal infectious diseases spread over hot and humid regions of the world. According to the WHO, it is ranked as the fourth dangerous infectious disease after pneumonia, HIV/AIDS and tuberculosis (NIAID, 2001). According to the latest WHO report, 91 countries are found as the endemic area from where 212 million new cases and 4,29,000 deaths reports have been recorded in 2015. There is a target to reduce the incidence and mortality of malaria globally up to 40% by 2020, 75% by 2025 and 90% by 2030 (WHO, 2017). Govt. of India (GoI) has also launched 'National Framework for Malaria Elimination in India 2016-2030' with the aim to eradicate malaria by 2030 (MoHFW GoI, 2016).

Although, there are numbers of drugs available for the treatment of malaria, there is an urgent need for new antimalarial drug discovery. It has been predicted that with the increased global warming, the geographical area covered by malaria vector, *Anopheles* species will increase and this will

cause wider transmission of malaria parasites (Laporta *et al.,* 2015). So, scientists and researchers are continuously working for versatile, robust alternatives to current antimalarial drugs to solve the different problems associated with the available antimalarial drugs (White, 2004; Travassos *et al.,* 2009; Guiguemde *et al.,* 2012; Taylor *et al.* 2004).

Among the various strategies adopted for drug development, natural product based drug discovery is one of the preferable ones (Newman *et al.,* 2016). Natural products have given numbers of important drug candidates for various critical diseases including malaria (Gurnani *et al.,* 2014). In the case of malaria, quinine and artemisinin are considered as two biggest milestones in the drug development of malaria and are the compounds of plant origin (Renslo *et al.,* 2013). Based on these two plant origin compounds, several highly useful semi synthetic and synthetic compounds have been derived. Some of them are already in use for the treatment of malaria and some of them are in the development (preclinical and clinical trial) stage (Fernández-Álvaro *et al.,* 2016). Other than these two plants, numbers of plants have been tested by scientists and researchers from different regions of the world including India for their antimalarial activity and some of them have shown potent activity (Willcox *et al.,* 2004). These plants are selected either randomly to explore the therapeutic potential or through studies guided by traditional information regarding their use in the treatment of fever. The ethno pharmacological approach has been recognized to give higher success rates for finding bioactive compounds (Carvalho *et al.,* 1991).

North-east India is one of the largest biodiversity regions of the country where people of different tribe and community use plant based traditional remedies to treat different diseases including malaria (Shankar *et al.,* 2015). Assam is one of the biodiversity rich States of the North-east India with huge

natural resources. Here numbers of different tribes and communities reside which practice traditional herbal remedies for the treatment of diseases and disorders. In Assam, numbers of indigenous plants have been reported to be useful as herbal remedies for the treatment of malaria disease (Namsa *et al.*, 2011; Gohain *et al.,* 2015). Although some of them used in these remedies are investigated scientifically to prove their antimalarial efficacy still there are some other traditional remedies which are yet to be investigated scientifically in the search of the new antimalarial drug candidate. These traditional remedies give a hope for finding out new drug candidate to help in the eradication program of malaria.

Materials and methods

Selection of plants materials

Three plants namely Tongloti (*Artemisia nilagirica* (Clarke) Pamp.), Dalim (*Punica granatum* L.) and Ram tulasi (*Elsholtzia blanda* Benth) were selected for preliminary screening of antimalarial activity from the preliminary filed survey reports in Upper Assam region. Different parts like leaves of *A. nilagirica* (Clarke) Pamp. and *E. blanda* Benth, and root of *P. granatum* L. were traditionally used to prepare antimalarial herbal remedies and people claim to get positive results from the treatments. The aforesaid plant parts were collected and processed according to standard procedure before grinded in to coarse powder by mechanical grinder. After that they were stored in airtight well closed container in dry place. The herbariums of the plants were prepared for identification and authentication by Botanical Survey of India, Shillong (Herbarium no. DU/DPS/2018-19/FS:01-04).

Extraction of plant materials

As in the preparation of traditional herbal remedies, mainly water is used as solvent or vehicle, the extraction of the powdered plant materials was carried out using hydro-alcoholic solvent system by cold maceration technique (Mandal *et al.,* 2015). Here about 100 gm of the powdered plant material for each plant parts were taken in 1 litre round bottom flasks and 500 ml of the solvents (hydro-alcoholic 1:1) were added to each of the flasks. The plant materials were kept in the solvents for 72 hours with occasional shaking. After completion of the extraction process, the solvents were first evaporated in rotary vacuum under reduced pressure and finally extracts were freeze dried by lyophilisation. The extracts were stored in desiccator under reduced pressure for further study.

In vitro antimalarial study

Preparation of standard and test samples

In the study chloroquine phosphate was used as standard drug and a stock solution of 100 µg/ml was prepared in dimethyl sulphoxide (DMSO) and incomplete medium (IRPMI). Similarly, for the test extracts, 1 mg/ml stock solution were prepared with DMSO and IRPMI medium.

Culture of malarial parasites

The antimalarial screening was carried out against both chloroquine sensitive (3D7) and chloroquine resistant (RKL-9) strain of *P. falciparum* by Giemsa stained slide method. In this method, the strains of *P. falciparum* are cultured at 37ºC and 5% CO_2 environment in RPMI (Roswell Park Memorial institute)-1640 medium supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate, gentamycin (40 mg/ml), Amphotericin-B (0.25 mg/ml) and 10% heat inactivated AB^+ serum (Trager *et al.,* 1976; Kumawat *et al.,* 2015).

Screening of the extracts

For antimalarial testing, the asynchronous parasites of *P. falciparum* were synchronized to obtain only the ring stage parasitized cells by the treatment of 5% D-sorbitol (Lambros *et al.,* 1976). For carrying out the assay, Parasitized blood is added to the wells of 96-well plate containing 100 µl of test sample (1 mg/ml conc.) serially diluted in the medium at different concentrations. The plates are incubated at 37^{o}C in environment of 5% CO_2 for 36-40 hour in CO_2 incubator. After incubation period, blood smears are prepared from each well in duplicates and stained with Giemsa stain. The schizonts stage (3 or more merozoites containing) are counted under light microscope and result is analyzed using by NonLin v.1.1 statistical software (Kumawat *et al.,* 2015; Pandey *et al.,* 2016).

Results and discussion

Extraction of plant materials

After extraction, the yield of the extract for the three plant parts were found as given in table 1.

Table 1: Yield of the three plant parts in hydroalcoholic (1:1) solvents

Sl. No.	Common name	Scientific name	Plant part used	Amount taken	% yield
1	Dalim	*Punica granatum* L.	Root	100 gm	5.19
2	Ram tulasi	*Elsholtzia blanda* Benth.	Leaf	100 gm	7.96
3	Tongloti	*Artemisia nilagirica*	Leaf	100 gm	6.77

	(Clarke) Pamp.

From the yield, it can be said that the parts of the three plants contain good quantity of different polar phytoconstiuents and yield is sufficient enough to carry out bioassay guided isolation of phytoconstituents.

In vitro antimalarial activity

The blood smears were prepared from the 96 well plates were observed under microscope after staining with Giemsa stain to count the number of schizonts present. The normal RBCs and parasites infected RBCs observed in the blood smears under microscope during the study were shown in fig 1. The *in vitro* antimalarial activity of the extracts were determined in the form of IC_{50} value and presented in table 2 along with the value for standard chloroquine phosphate.

Table 2: IC_{50} values of the plant extracts

Sl. No.	Samples	IC_{50} (µg/ml)	
		CQ-S *Pf*3D7	CQ-R *Pf*RKL-9
1	Standard drug (Chloroquine phosphate)	0.708	0.803
2	*P. granatum* L. extract	5.705	6.221
3	*E. blanda* Benth. Extract	4.172	5.604
4	*A. nilagirica* (Clarke) Pamp. Extract	3.274	5.373

Out of the three extracts, the extract of *A. nilagirica* (Clarke) Pamp. has shown good antimalarial activity (IC_{50}=3.274 µg/ml & 5.373 µg/ml) against

both chloroquine sensitive and resistant strain respectively. Although results were not comparable with standard drug chloroquine, but at extract level the results were satisfactory. Generally, extract having IC_{50} value less than 5 µg/ml is considered as highly active and can proceed for further study (Ouattara *el al.,* 2014). The parameters for selecting an extract as active, intermediate or in active in this study are given in table 3 (Lima *et al.,* 2015).

Table 3: Categorization of activity based on IC_{50} values

IC_{50} range	Remarks
<10 µg/ml	Active
10-25 µg/ml	Intermediate
>25 µg/ml	Inactive

From the comparison, all the three extracts were come under active category against both the sensitive and resistant strain of *P. falciparum.* As the plant parts are used with water in the form of decoction and solution in their respective traditional practices, this study validate their use as antimalarial herbal remedies. The plant parts might contain some polar phytoconstituents which are responsible for the antimalarial activity. Further bioassay guided fractionation and isolation is necessary to find out the compounds having potent antimalarial activity and can serve as a lead candidate for drug development process.

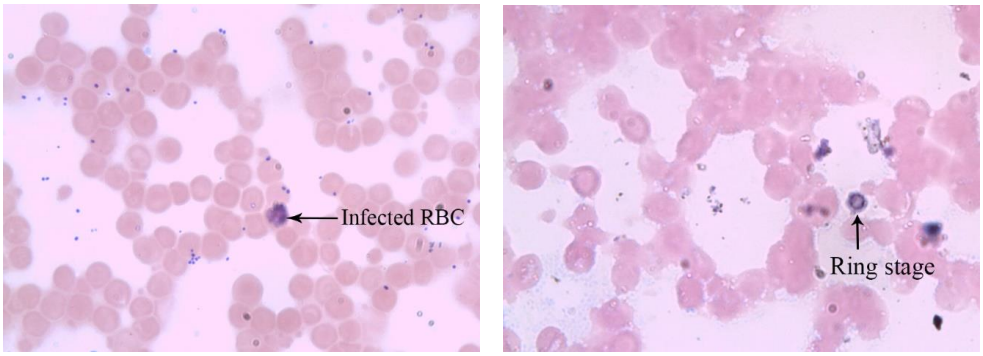

Fig 1: Infected RBC and ring stage of the parasite under microscope at 100X

Conclusion

From the above study, the traditional use of the plant materials from the selected three plants have been validated by the *in vitro* study. Although the activities were not comparable with the standard drug chloroquine phosphate but it gave a direction towards identification of new phytoconstituents with antimalarial activity. These findings may later help in developing new semisynthetic derivatives from the lead molecules with improved efficacy and safety. Here only hydro-alcoholic extracts were screened based on their traditional practice methods. But extraction of the plant materials with other nonpolar to mid polar solvent is continuing as phytoconstiuents present in the nonpolar or mid polar region might also have better antimalarial activities.

Acknowledgement

Authors acknowledge the financial assistance received from University Grant Commission under UGC-SAP (DRS-I) programme to carry out the research work and the local practitioners of Dhemaji, Charaideo and Sivasagar District for sharing the information regarding their herbal remedies.

Conflict of interest

There is no conflict of interest.

References

Alkadi HO (2007). Antimalarial drug toxicity: A review. *Chemotherapy*, 53(6):385-391. doi:10.1159/000109767

Azmir J, Zaidul ISM, Rahman MM, *et al*. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *J Food Eng*, 117(4):426-436. doi:10.1016/j.jfoodeng.2013.01.014

Carvalho LH, Krettli AU (1991). Antimalarial chemotherapy with natural products and chemically defined molecules. *Mem Inst Oswaldo Cruz*, 86 Suppl 2:181-184. doi:10.1590/S0074-02761991000600041

Fernández-Álvaro E, Hong WD, Nixon GL, *et al.* (2016). Antimalarial Chemotherapy: Natural Product Inspired Development of Preclinical and Clinical Candidates with Diverse Mechanisms of Action. *J Med Chem*, 59(12):5587-5603. doi:10.1021/acs.jmedchem.5b01485

Gohain N, Prakash A, Gogoi K, *et al.* (2015). An ethnobotanical survey of anti-malarial plants in some highly malaria affected districts of Assam. *Int J Pharm Pharm Sci*, 7(9):147-152.

Guiguemde WA, Guy RK (2012). An all-purpose antimalarial drug target. *Cell Host Microbe*, 11(6):555-557. doi:10.1016/j.chom.2012.05.008

Gurnani N, Mehta D, Gupta M, Mehta BK (2014). Natural Products: Source of Potential Drugs. *Afr J Basic Appl Sci*, 6(6), 171-186.

Kumawat MK, Chetia D (2015). Synthesis, antimalarial activity evaluation and molecular docking studies of some novel dispiro-1,2,4,5-tetraoxanes. *Bangladesh J Pharmacol*, 10(4):917-923. doi:10.3329/bjp.v10i4.24532

Lambros C, Vanderberg JP (1979). Synchronization of Plasmodium falciparum erythrocytic stages in culture. *J Parasitol*, 65(3):418-420.

Laporta GZ, Linton YM, Wilkerson RC, et al. (2015). Malaria vectors in South America: Current and future scenarios. *Parasites and Vectors*, 8(1). doi:10.1186/s13071-015-1038-4

Lima RBS, Rocha Silva LF, Melo MRS, *et al*. (2015). *In vitro* and *in vivo* anti-malarial activity of plants from the Brazilian Amazon. *Malar J*, 14(1). doi:10.1186/s12936-015-0999-2

Mandal SC, Mandal V, Das AK (2015). Classification of Extraction Methods. In: Essentials of Botanical Extraction. Cambridge: Academic Press, pp.83-136. doi:10.1016/b978-0-12-802325-9.00006-9

Ministry of Health & Family Welfare, Government of India (MoHFW, GoI) (2016). National Framework for Malaria Elimination in India (2016-2030), Directorate of National Vector Borne Disease Control Programme (NVBDCP), Directorate General of Health Services (DGHS). https://apps.who.int/iris/bitstream/handle/10665/246096/national_framework_malaria_elimination_india_2016_2030.pdf?sequence=1&isAllowed=y (27th February, 2019).

Namsa ND, Mandal M, Tangjang S (2011). Anti-malarial herbal remedies of northeast India, Assam: An ethnobotanical survey. *J Ethnopharmacol*, 133(2):565-572. doi:10.1016/j.jep.2010.10.036

National Institute of Allergy and Infectious Diseases (NIAID) (2001). Global health research plan for HIV/AIDS, malaria and tuberculosis. *WHO.* https://www.tballiance.org/downloads/newsbriefs/niaid_report.pdf (9th March, 2019)

Newman DJ, Cragg GM (2016). Natural Products as Sources of New Drugs from 1981 to 2014. *J Nat Prod,* 79(3):629-661. doi:10.1021/acs.jnatprod.5b01055

Ouattara LP, Sanon S, Mahiou-Leddet V, *et al.* (2014). *In vitro* antiplasmodial activity of some medicinal plants of Burkina Faso. *Parasitol Res,* 113(1):405-416. doi:10.1007/s00436-013-3669-8

Pandey AK, Sharma S, Pandey M, *et al.* (2016). 4, 5-Dihydrooxazole-pyrazoline hybrids: Synthesis and their evaluation as potential antimalarial agents. *Eur J Med Chem,* 123:476-486. doi:10.1016/j.ejmech.2016.07.055

Renslo AR (2013). Antimalarial drug discovery: From quinine to the dream of eradication. *ACS Med Chem Lett,* 4(12):1126-1128. doi:10.1021/ml4004414

Shankar R, Deb S, Sharma BK (2015). Traditional Healing Practices in North East India. *Indian J Hist Sci,* 50 (2):324-332.

Taylor WR, White NJ (2004). Antimalarial drug toxicity: a review. *Drug Saf,* 27(1):25-61. doi:10.2165/00002018-200427010-00003

Trager W, Jensen JB (1976). Human malaria parasites in continuous culture. Science, 193(4254):673-675. doi:10.1126/science.781840

Travassos MA, Laufer MK (2009). Resistance to antimalarial drugs: Molecular, pharmacologic, and clinical considerations. *Pediatr Res*, 65(SUPPL. 5). doi:10.1203/PDR.0b013e3181a0977e

Willcox M, Bodeker G, Rasoanaivo P, *et al*. (2004). Traditional Medicinal Plants and Malaria, CRC Press.

White NJ (2004). Antimalarial drug resistance. *J Clin Invest*, 113(8):1084-1092. doi:10.1172/JCI21682

World Malaria Report 2016 (2017). *WHO*. http://www.who.int/malaria/publications/world-malaria-report 2016/report/en/ (3rd March, 2019).

Zhou B, Wu Y, Dalal S, et al (2017). Nanomolar antimalarial agents against chloroquine-resistant plasmodium falciparum from medicinal plants and their structure-activity relationships. *J Nat Prod*, 80(1):96-107. doi:10.1021/acs.jnatprod.6b00744

How to cite this article:

Gogoi N and Chetia D. Preliminary validation of ethnomedicinal plants used in the preparation of antimalarial herbal remedies by *in vitro* screening method, *Curr Trend Pharm Res,* 2019, 6 (1): 63-75.