Innovations in Pharmaceuticals and Pharmacotherapy

www.innpharmacotherapy.com

eISSN: 2321–323X

Research Article

Evaluation of *in vitro* and *in vivo* antimalarial activity of *Hymenodictyon excelsum* bark extracts

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Abstract

The present study was designed to evaluate *in vitro* and *in vivo* antimalarial activity of different extracts of *Hymenodictyon excelsum* bark which is used in the treatment of malarial fever as a febrifuge. The preliminary phytochemical screening of different extracts of *Hymenodictyon excelsum* bark showed the presence of alkaloids, flavonoids, glycosides, steroids, tannins and terpenoids. The ethyl acetate extract of Hymenodictyon excelsum has shown dose dependent percentage Inhibition of schizont maturation of Plasmodium falciparum and the highest in vitro antimalarial activity by schizont maturation inhibition assay indicated by IC50 = 35.72 µg/ml. Percentage parasitaemia with 50, 100 and 200 mg/kg/day doses of ethyl acetate extract of Hymenodictyon excelsum ranged from 8.33 ± 0.29 to 6.33 ± 0.29 and maximum percentage chemosuppression was observed with 200 mg/kg/day dose which was highly significant (86.63 %) as compared to standard (85.93%). The maximum survival of mice was observed with 200 mg/kg/day of ethyl acetate extract of *Hymenodictyon excelsum* (28 days) whereas, less significant results were recorded with petroleum ether extract (12.00 ± 1.00 days). Thus, it can be concluded that the ethyl acetate extract of *Hymenodictyon excelsum* could be possessed antimalerial activity.

Keywords: Hymenodictyon excelsum bark, antimalarial activity, schizont maturation inhibition assay.

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1. Introduction

A large number of herbal medicines are presumed to have admirable therapeutic value and can be employed for the management of many ailments. In natural medicine, different local herbs are used, in single or in combined form, to treat different types of infectious diseases, with great success. Even though the exercise of these herbs has a sound practice, and their therapeutic uses are well known to local people, their place has yet to be modernized in therapeutics, using the recent methods. Scientific studies are therefore necessary to evaluate their effectiveness to broaden the scope of these herbs. Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug synthesis because of the unmatched availability of chemical diversity [1].

The term malaria originates from medieval italian: *mala aria* - "bad air" and the disease was formerly called ague or marsh fever due to its association with swamps [2]. The resistance of *Plasmodium falciparum* to the conventional antimalarial drugs is the key factor responsible for increasing mortality and morbidity of malaria [3-5]. The drug resistance usually occurs due to change in the drug accumulation [6] or reduced affinity of the drug on target [7-8]. The resistance occurs when the drug concentrations are sufficient to reduce the susceptible parasite population but inhibit less or do not inhibit multiplication of the mutants [9]. Increasing multidrug resistance *Plasmodium falciparum* in many parts of the world has aggravated the problem of deciding which antimalarial to use, particularly in countries where *Plasmodium falciparum* has developed resistance to chloroquine, mefloquine, primaquine, antifolates such as fansidar (sulphadoxine-pyrimethamine)

and, to some extent, quinine which previously was effective in the treatment of severe and complicated malaria [10].

Chloroquine has been the basic drug in the management of malaria from long decades. This cost effective and safe drug has become unsuccessful for management of falciparum malaria in many parts of the world due to development of resistance by the malaria parasite. In india, chloroquine resistance was reported for the first time in Assam [11] in 1973 which was followed by several reports of chloroquine resistance especially from Orissa, North Eastern states, Madhya Pradesh and Gujarat [12-15]. The study of the use of plants by indigenous peoples followed by phytochemical, preclinical and clinical studies is an important approach toward the discovery and development of traditional medicines [16].

It was observed from ayurvedic literature and ethnopharmacological studies, that various plants are very useful in treating malaria but no scientific investigation has been done in such direction. Literature survey reveals that *Hymenodictyon excelsum* bark exhibits wide spectrum of medicinal value and they are used in the treatment of malaria. So, it's imperative to evaluate it scientifically and investigate its active phytoconstituents. The objective of present study was to evaluate in vitro and in vivo antimalarial activity of *Hymenodictyon excelsum* (Rubiaceae) bark.

2. Material and methods

Giemsa stain, HEPES and quinine dihydrochloride were purchased from Sigma Chemicals, USA. Chloroquine phosphate base was available from Universal Medikit, Nagpur. All other chemicals and reagents used for study in the present investigation were of analytical grade.

Swiss albino mice (20-25 g) of either sex were housed under standard laboratory condition of 12:12 h light/dark cycle in a temperature-controlled (24 ± 10ºC) environment with *ad libitum* access to rodent chow (Lipton, India) and water. All experimental protocols were approved by Institutional Animal Ethics Committee (IAEC). Committee Constituted for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi, India (IAEC approval No. 536/02/C/CPCSEA dated 20.01.02).

Collection and authentication of *Hymenodictyon excelsum*

Hymenodictyon excelsum (Rubiaceae) bark was collected from the forest of Itarasi, Madhya Pradesh, India. Herbarium specimens were prepared and submitted at Department of Botany, Bhawbhuti Mahavidyalaya, Aamgaon, Dist. Gondia. The plant specimens were authenticated by Dr. Madhusudan Bhuskute, Principal and HOD, Department of Botany, Bhawbhuti Mahavidyalaya, Aamgaon.

Extraction of *Hymenodictyon excelsum*

Bark of *Hymenodictyon excelsum* was extracted successively in order of increasing polarity of solvents such as petroleum ether (PE), ethyl acetate (EA), acetone (AC) and ethanol (ET). Hydro-alcoholic (HA) extract [absolute ethanol: distilled water (1:1)] was obtained by maceration after 15 days. All the extracts were collected, filtered through Whatman filter paper (No. 44), concentrated over thermostat water bath. Dried extracts were kept in tight container, preserve in BOD incubator till further use.

Preliminary phytochemical screening

All the extracts were screened for presence of phytoconstituents viz. alkaloids, flavonoids, tannins, steroids, saponins, triterpenoids, proteins and sugars [17].

Acute toxicity study of extracts according to OECD guidelines

Acute oral toxicity study was carried out in mice in stepwise procedure using fixed dose as per OECD-420 guidelines.

Evaluation of *in vitro* antimalarial activity of different extracts of *Hymenodictyon excelsum* by schizont maturation inhibition assay

Different extracts of *Hymenodictyon excelsum* were evaluated for antimalarial activity by schizont maturation inhibition assay. Parasites were incubated with different test extracts and then parasitemia of control and treated groups are compared by counting Giemsa stained parasites using light microscopy. This technique relies on a morphological criterion of response. This is a reliable technique because in this method parasite inhibition can be seen visually.

Strain of *Plasmodium falciparum* (MRC-Pf-596) was obtained from National Institute of Malaria

Research (NIMR), New Delhi and was cultured and maintained by candle jar method of Trager and Jensen (1976) in vitro. The medium used was RPMI-1640 (Sigma-Alderich) supplemented with 25 mM HEPES, 0.2 % $NaHCO_3$ and 10 % human serum.

Stock solutions of test extarcts and standard were prepared by dissolving known quantities of PE, EA, AC, ET, HA extracts and quinine dihydrochloride in DMSO. These were further diluted with RPMI-1640 to achieve the required concentration before testing with the culture.

Each concentration of extracts and quinine dihydrochloride and solvent (negative control) were run in triplicate 96 well microtitre plates. Each well contained 20 µl of diluted extract and 200 µl RPMI 1640 supplemented with parasitized RBCs (1:10 ratio of extract and medium). These plates were incubated at 37º C in the candle jar. After 24 h of incubation, these smears from each well were prepared and stained with Giemsa stain. The morphological features of erythrocytic blood stages of parasites including rings, tropozoites and schizonts were observed under microscope. The number of parasitized RBCs per 100/500 cells were counted and IC_{50} value of each extract was estimated. IC_{50} (50 % inhibiting concentration) is the drug concentration corresponding to 50 % inhibition of schizont development as compared to control. Percent schizont maturation inhibition was calculated by using this equation:

% schizont maturation inhibition = 100 X A-B/A

A - Average schizont maturation in untreated control well
B - Average schizont maturation in extract/ standard treated well

IC_{50} value was determined by plotting a graph between various concentrations of extracts and percentage of schizont maturation inhibition corresponding to that concentration [18-20].

Evaluation of *in vivo* antimalarial activity of different extracts of *Hymenodictyon excelsum* by Peter's 4 days suppressive test

The test described by Peter's for antimalarial activity against a Chloroquine sensitive *Plasmodium falciparum* in albino mice was used for evaluating in-vivo antimalarial activity of different extracts of *Hymenodictyon excelsum* under study.

Inoculation of experimental animals with parasite

The Chloroquine sensitive *Plasmodium falciparum* (MRC-Pf-435) *was obtained from* National Institute of Malaria Research (NIMR), New Delhi and maintained as per procedure described by Knight and Peters (1980) in mice. Inoculum was prepared by diluting the blood obtained from the donar mouse previously infected with *Plasmodium falciparum* with isotonic saline in proportions indicated by erythrocyte count and percentage parasitaemia. The inoculums consisted $5x10^7$ parasitized erythrocytes and 29% parasitaemia. Mice were infected by intraperitoneal injection of 0.2 ml of above inoculums on day 0 [21].

Determination of schizonticidal activity [19, 21-22].

The animals were randomly divided into 17 groups of 4 mice each shortly after the inoculation.

Group 1: treated with saline *p.o.* (Control)
Group 2: treated with PE extract (50 mg/kg *p.o.*)
Group 3: treated with PE extract (100 mg/kg *p.o.*)
Group 4: treated with PE extract (200 mg/kg *p.o.*)
Group 5: treated with EA extract (50 mg/kg *p.o.*)
Group 6: treated with EA extract (100 mg/kg *p.o.*)
Group 7: treated with EA extract (200 mg/kg *p.o.*)
Group 8: treated with AC extract (50 mg/kg *p.o.*)
Group 9: treated with AC extract (100 mg/kg *p.o.*)
Group 10: treated with AC extract (200 mg/kg *p.o.*)
Group 11: treated with ET extract (50 mg/kg *p.o.*)
Group 12: treated with ET extract (100mg/kg *p.o.*)
Group 13: treated with ET extract (200 mg/kg *p.o.*)
Group 14: treated with HA extract (50 mg/kg *p.o.*)
Group 15: treated with HA extract (100 mg/kg *p.o.*)
Group 16: treated with HA extract (200 mg/kg *p.o.*)
Group 17: treated with Chloroquine 5 mg/kg *p.o.* (Standard)

The animals were grouped as stated above and treated with PE, EA, AC, ET and HA extracts of *Hymenodictyon excelsum.*

Estimation of percentage parasitaemia:

$$\% \text{ Parasitaemia} = \frac{\text{Parasitized RBC}}{\text{Parasitised RBC + Non parasitized RBC}} \times 100$$

The treatments were given from day 0 (day of inoculation) for four consecutive days (day 0 to day 3). On the fifth day (day 4), thin films were made from the tail venepuncture of each mouse and the parasitaemia level was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average % chemosuppression was calculated as:

Estimation of percentage chemosuppression:

Average % Chemosuppression = 100 X(A-B)/A

Where,
A = Average % parasitaemia in control group
B = Average % parasitaemia in test group

Evaluation of schizonticidal activity in established infection (Curative or Rane test)

Evaluation of schizonticidal activity in established infection (Curative or Rane test) was done by the method described by Ryley and Peter (1970). The mice were injected intraperitoneally with standard inoculums of 1 x 10^7 *Plasmodium falciparum* infected erythrocytes on the first day (day 0). Seventy two hours later, the mice were divided into 16 groups of 4 mice each. The groups were orally administered with the respective treatment as stated above once daily for 5 days.
Thin films stained with Giemsa stain were prepared from the tail venepuncture of each mouse daily for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined by finding the average survival time (days) of the mice in each group over a period of 28 days (day 0 to day 27) [23-24].

Mean survival time = Sum of days of survival of animals (groups)/ Total number of animals in the group

Statistical analysis

Data were presented as mean ± SD. Statistical analysis was carried out using one way ANOVA using Graph Pad Prism Software Version 6.04. Differences were considered statistically significant at $P \leq 0.05$.

3. Result and discussion

Different extracts of *Hymenodictyon excelsum* have shown presence of alkaloids flavonoids, glycosides, steroids, tannins and triterpenoids. Presence of sterols and triterpenoids were confirmed when sprayed with p-anisaldehyde sulfuric acid reagent, it showed red and green colour. Detection of alkaloids was confirmed by spraying Dragendroff's reagent, showed orange colour spots. Tannins were observed blue - greenish color when sprayed with 5 % $FeCl_3$ solution.
Different extracts of *Hymenodictyon excelsum* were evaluated for in vitro antimalarial activity by schizont maturation inhibition assay. This technique relies on a morphological criterion of response. This is a reliable technique because in this method parasite inhibition can be seen visually [18-20]. Less IC_{50} value indicates higher antimalarial potency of the extract. The ethyl acetate extract of *Hymenodictyon excelsum* has shown dose dependent % Inhibition of schizont maturation of *Plasmodium falciparum* and the highest in vitro antimalarial activity by schizont maturation inhibition assay indicated by IC_{50} = 35.72 µg/ml. Ethanolic and hydro-alcoholic extracts of HE showed less significant % schizont inhibition with IC_{50} = 61.93 and 91.35 µg/ml, respectively. All other extracts of *Hymenodictyon excelsum* had showed non-significant activity with IC_{50} > 100 µg/ml (Table 1; Figure 1). The results were compared with Quinine dihydrochloride as standard.

Before subjecting the extracts for in vivo antimalarial activity, toxicity studies were carried out as per OECD guidelines. The results of acute oral toxicity studies revealed that all extracts of *Hymenodictyon excelsum* were found to be nontoxic up to 2000 mg/ kg dose *p. o.* There were no significant changes in the gross behavior, signs of toxicity and mortality during the study.

The *in vivo* antimalarial activity of different extracts of *Hymenodictyon excelsum* was evaluated against *Plasmodium falciparum* infected mice by Peter's 4 days suppressive test [21-22]. Values for % parasitaemia and % chemosuppression are inversely proportional to

each other. Lower values for percentage parasitaemia indicate higher percentage chemosuppression which shows better antimalarial potency.

Table 1: Effect of different extracts of *Hymenodictyon excelsum* on schizont maturation of Plasmodium falciparum

Extracts	Dose (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Hymenodictyon excelsum PE	10	4.51 ± 0.74	> 100
	25	5.56 ± 0.38	
	50	6.51 ± 0.25	
	100	9.28 ± 0.71	
Hymenodictyon excelsum EA	10	35.99 ± 0.53	35.72***
	25	43.01 ± 0.75	
	50	61.38 ± 0.89	
	100	81.08 ± 0.91	
Hymenodictyon excelsum AC	10	6.49 ± 0.43	> 100
	25	7.65 ± 0.47	
	50	13.64 ± 1.95	
	100	26.50 ± 1.89	
Hymenodictyon excelsum ET	10	16.23 ± 0.87	61.93**
	25	26.98 ± 1.71	
	50	54.48 ± 0.45	
	100	66.90 ± 1.42	
Hymenodictyon excelsum HA	10	12.52 ± 0.60	91.35
	25	19.14 ± 0.26	
	50	32.84 ± 0.62	
	100	53.40 ± 1.17	
Standard Quinine dihydrochloride	0.005	40.48 ± 0.28	0.0096
	0.01	45.33 ± 0.30	
	0.015	65.07 ± 0.42	
	0.020	83.14 ± 2.25	

N = 3, Values are expressed in Mean ± SD. * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

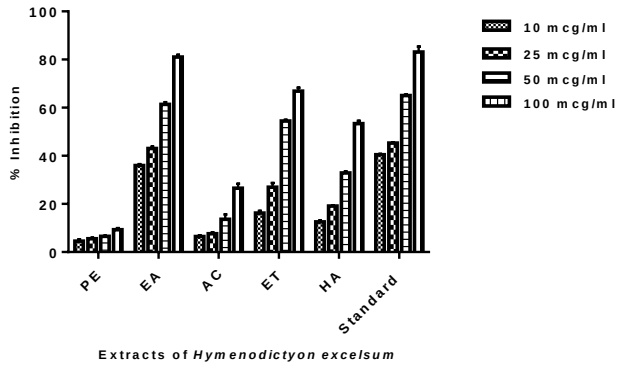

Figure1: Effect of different extracts of Hymenodictyon excelsum on schizont maturation of Plasmodium falciparum

The average percentage parasitaemia and percentage chemosuppression of different extracts of *Hymenodictyon excelsum* were determined. Chloroquine was used a reference standard. Percentage parasitaemia with 50, 100 and 200 mg/kg/day doses of ethyl acetate extract of *Hymenodictyon excelsum* ranged from 8.33 ± 0.29 to 6.33 ± 0.29 and maximum percentage chemosuppression was observed with 200 mg/kg/day dose which was highly significant (86.63 %) as compared to standard (85.93 %). The results of all other extracts were non-significant (Table 2; Figure 2).

Table 2: Evaluation of in vivo schizonticidal activity of different extracts of Hymenodictyon excelsum

Extracts	Dose (mg/kg/ day)	% Parasitaemia	% Chemo-suppression
Hymenodictyon excelsum PE	50	27.33 ± 0.29	42.26
	100	26.33 ± 0.29	44.37
	200	26.16 ± 0.29	44.73
Hymenodictyon excelsum EA	50	8.33 ± 0.29	82.40***
	100	7.33 ± 0.29	84.51***
	200	6.33 ± 0.29	86.63***
Hymenodictyon excelsum AC	50	24.33 ± 0.29	48.60
	100	24.33 ± 0.29	48.60
	200	23.33 ± 0.29	50.71
Hymenodictyon excelsum ET	50	18.5 ± 0.50	60.91
	100	18.17 ± 0.29	61.61
	200	17.17 ± 0.29	63.72
Hymenodictyon excelsum HA	50	23.33 ±0.29	50.71
	100	22.33 ± 0.29	52.82
	200	21.17 ± 0.29	55.27
Standard Chloroquine	5	6.67 ± 0.29	85.93***
Control Normal Saline solution	0.2 ml	47.33 ± 2.52	-

N = 4, Values are expressed in Mean ± SD. * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

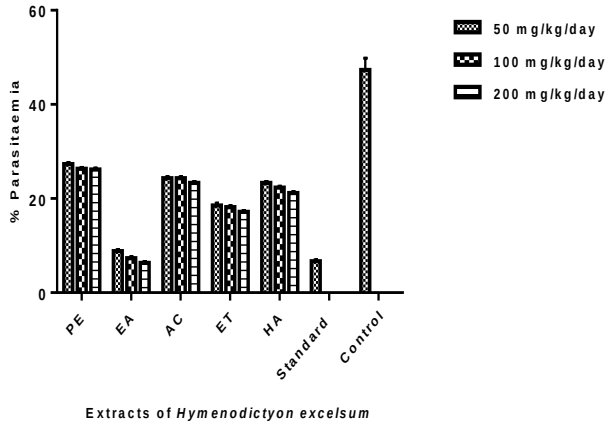

Figure 2: % Parasitaemia in mice receiving different extracts of Hymenodictyon excelsum

Also, evaluation of schizonticidal activity of all extracts of *Hymenodictyon excelsum* in established infection (Curative or Rane test) was carried out by the method described by Ryley and Peter (1970). The mean survival time for each group was determined by finding the average survival time (days) of the mice in each group over a

period of 28 days (day 0 to day 27) [23-24]. More the Mean survival time (MST), more is the efficacy of the extract. The least Mean survival time (MST) of 8 days was recorded for the control group that was left untreated. The mice in the standard group treated with Chloroquine recorded MST of 28 days. The maximum survival of mice was observed with 200 mg/kg/day of ethyl acetate extract of *Hymenodictyon excelsum* (28 days) whereas, less significant results were recorded with petroleum ether extract (12.00 ± 1.00 days) (Table 3; Figure 3).

Table 3: Mean survival time of mice receiving different extracts of *Hymenodictyon excelsum*

Extracts	Dose (µg/ml)	Mean survival time (days)
Hymenodictyon excelsum PE	50	11.33 ± 1.16
	100	11.68 ± 2.89
	200	12.00 ± 1.00
Hymenodictyon excelsum EA	50	26.68 ± 0.58***
	100	26.33 ± 1.53***
	200	28.00 ± 0.00***
Hymenodictyon excelsum AC	50	13.68 ± 1.53
	100	12.00 ± 2.00
	200	12.00 ± 1.00
Hymenodictyon excelsum ET	50	15.33 ± 0.58
	100	16.68 ± 0.58
	200	17.67 ± 0.58
Hymenodictyon excelsum HA	50	15.68 ± 1.53
	100	13.68 ± 1.53
	200	14.68 ± 1.16
Standard Chloroquine	5	28 ± 0.00***
Control Normal Saline solution	0.2 ml	7.66 ± 0.58

N = 4, Values are expressed in Mean ± SD. * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

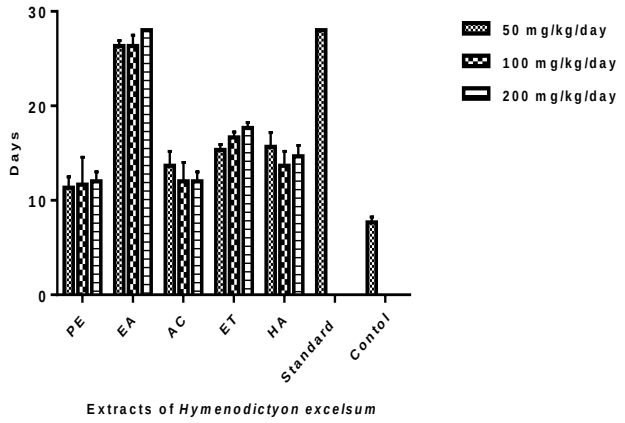

Figure 3: Mean survival time of mice receiving different extracts of *Hymenodictyon excelsum*

Conclusion

Based on the results obtained it can be concluded that, ethyl acetate (EA) extracts of *Hymenodictyon excelsum* possess significant in vitro and *in vivo* antimalarial activity. Isolation of bioactive phytoconstituents from these extracts for the development of new compounds for the treatment of malaria will be carried out in future.

Acknowledgement

The authors are thankful to Principal, Sharad Pawar College of Pharmacy, Nagpur for providing necessary facilities to carry out the research work.

References

1. Cos, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. Journal of ethnopharmacology, 106(3), 290-302.
2. Cox F. History of human parasitology. Clinical Microbiol Rev. 2002; 15 (4): 595-612.
3. Ridley, R. G. (2002). Medical need, scientific opportunity and the drive for antimalarial drugs. Nature, 415(6872), 686-693.
4. Ridley, R. G. (2002). Medical need, scientific opportunity and the drive for antimalarial drugs. Nature, 415(6872), 686-693.
5. Sowunmi, A., Oduola, A. M. J., Ogundahunsi, O. A. T., & Salako, L. A. (1998). Comparative efficacy of chloroquine plus chlorpheniramine and pyrimethamine/sulfadoxine in acute uncomplicated falciparum malaria in Nigerian children. Transactions of the Royal Society of Tropical Medicine and Hygiene, 92(1), 77-81.
6. White, N. J. (1998). Drug resistance in malaria. British Medical Bulletin, 54(3), 703-715.
7. Foote, S. J., & Cowman, A. F. (1994). The mode of action and the mechanism of resistance to antimalarial drugs. Acta tropica, 56(2), 157-171.
8. Ward, S. A., Bray, P. G., Mungthin, M., & Hawley, S. R. (1995). Current views on the mechanisms of resistance to quinoline-containing drugs in Plasmodium falciparum. Annals of tropical medicine and parasitology, 89(2), 121-124.
9. Chawira, A. N., & Warhurst, D. C. (1987). The effect of artemisinin combined with standard antimalarials against chloroquine-sensitive and chloroquine-resistant strains of Plasmodium falciparum in vitro. The Journal of tropical medicine and hygiene, 90(1), 1-8.
10. Olliaro, P. L., & Trigg, P. I. (1995). Status of antimalarial drugs under development. Bulletin of the World Health Organization, 73(5), 565.

11. Sehgal, P. N., Sharma, M. I. D., Sharma, S. L., & Gogai, S. (1973). Resistance to chloroquine in falciparum malaria in Assam state, India. J Commun Dis, 5, 175-80.
13. Valecha N (1996) Resistant Malaria; Frontiers; in Padeiatrics (eds) H P S Sachdev and Panna Chaudhry (New Delhi: Jaypee Publications) pp 116–138.
14. Sharma, V. P. (1999). Current scenario of malaria in India. Parassitologia, 41(1-3), 349-353.
15. Sharma V P (2000) Status of drug resistance in malaria in India; in Multi-drug resistance in emerging and re-emerging diseases (ed.) R C Mahajan (Delhi: Narosa Publications) pp 191–202.
16. Willcox, M., Burford, G., & Bodeker, G. (2004). An overview of ethnobotanical studies on plants used for the treatment of malaria. Traditional Medicinal Plants and Malaria, 2004.
17. Trease, G.E., Evans, W.C., (1989). Pharmacognosy. Brailliar Tiridel can, 13th ed. Macmillian Publishers, 115
18. Bhat, G. P., & Surolia, N. (2001). In vitro antimalarial activity of extracts of three plants used in the traditional medicine of India. The American journal of tropical medicine and hygiene, 65(4), 304-308.
19. Verma, G., Dua, V. K., Agarwal, D. D., & Atul, P. K. (2011). Anti-malarial activity of Holarrhena
12. Misra, S. P. (1996). In-vivo resistance to chloroquine and sulfa-pyrimethamine combination in Plasmodium falciparum in India. Proc Natl Acad Sci India, 66, 123-128.
antidysenterica and Viola canescens, plants traditionally used against malaria in the Garhwal region of north-west Himalaya. Malar J, 10, 20.
20. Chandel, S., & Bagai, U. (2010). Antiplasmodial activity of Ajuga bracteosa against Plasmodium berghei infected BALB/c mice. Indian Journal of Medical Research, 131(3), 440.
21. Nafiu, M. O., Abdulsalam, T. A., & Akanji, M. A. (2013). Phytochemical Analysis and Antimalarial Activity Aqueous Extract of Lecaniodiscus cupanioides Root. Journal of tropical medicine, 2013.
22. Peters, W. (1965). Competitive relationship between Eperythrozoon coccoides and Plasmodium berghei in the mouse. Experimental parasitology, 16(2), 158-166.
23. Ryley, J. F., & Peters, W. (1970). The antimalarial activity of some quinolone esters. Ann. Trop. Med. Parasit., 64(2), 209-22.
24. I Okokon, J. E., Ettebong, E., & Antia, B. S. (2008). In vivo antimalarial activity of ethanolic leaf extract of Stachytarpheta cayennensis. Indian journal of pharmacology, 40(3), 111.