Screening of Natural/Synthetic Compounds for Antimalarial Activity

Plants have been used as a traditional medicine for the treatment of malaria. Plants may provide drugs directly such as quinine from cinchona bark or they may provide template molecules on which to base further new structures by organic synthesis—artemisinin from *Artemisia annua*. At the NIMR, efforts are being made to do primary screening of crude extracts of plant products to screen different fractions isolated from various parts of plants and to isolate pure compounds having antimalarial properties.

Primary Screening of Plant Products

Aqueous extracts of *Azadirachta indica* (bark), *Phyllanthus niruri* (whole plant) and *Ocimum sanctum* (leaves) were tested *in vivo* against *P. berghei* following Peter's 4-day test. Antimalarial effect of three medicinal plants tested is shown in Fig. 27 (Usha Devi *et al* 2001).

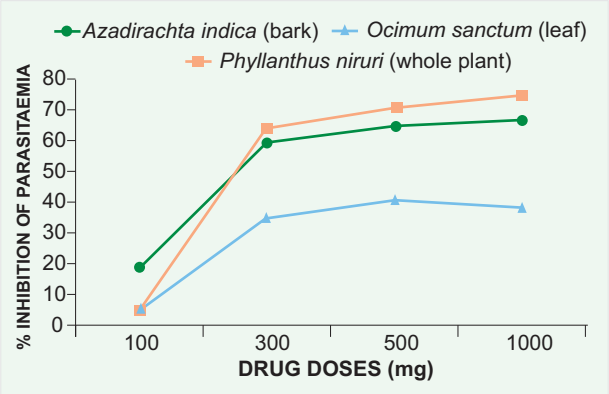


Fig. 27: *In vivo* antimalarial effect of three medicinal plants (aqueous extracts)

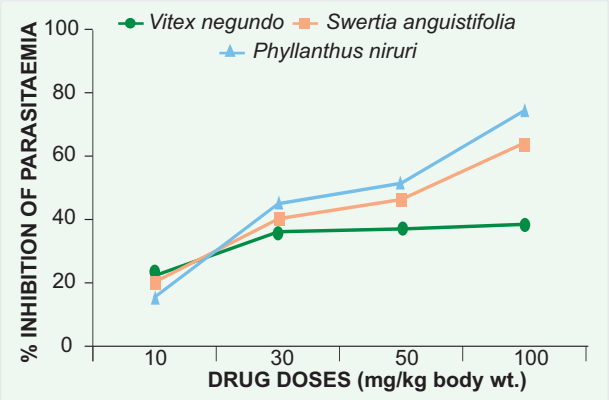


Fig. 28: *In vivo* antimalarial effect of three medicinal plants (50% ethanol extracts)

Ethanol extract (50%) of nine medicinal plants were tested *in vitro* for their antimalarial activities using CQ sensitive isolate. IC_{50} values ranged from 0.3–70.0 µg/ml. Some of these extracts, showing encouraging results with *in vitro* system, had also been tested *in vivo* against *P. berghei* following Peter's 4-day test. The study showed that, *Phyllanthus niruri* and *Swertia anguistifolia* plants have good antimalarial properties whereas *Vitex negundo* had less effect (Fig. 28).

Screening of Fractions

Andrographis paniculata

Andrographis paniculata is widely used as a folk medicine in China and southeast Asia. Leaves of *Andrographis paniculata* (local name *Bhuineem*) has been extensively used as a traditional medicine for the treatment of symptomatic malaria by the tribal population of Bastar district, Madhya Pradesh, India (Dua *et al* 1999). Therefore, a study was undertaken to investigate the antimalarial activity of this plant. The roots from the dried plants (Source: Gurukul University, Hardwar) were separated, washed with distilled water, dried under shade and solvent partitioned with four different polarity solvents—petroleum ether, methanol, chloroform and water using soxhlet apparatus. The four fractions so obtained, namely AG-1, AG-2 AG-3 and AG-4 were screened *in vitro* for schizontocidal activity (Fig. 29). Since AG-3 possessed promising antimalarial activity, it was selected for further studies.

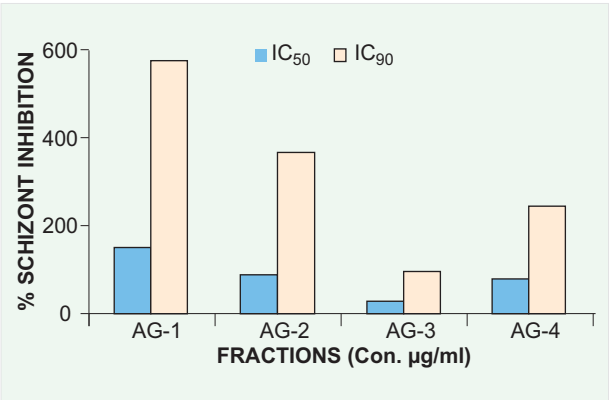
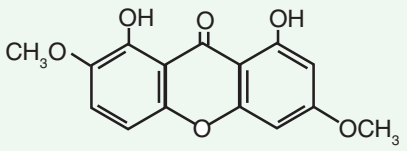
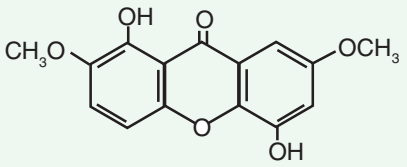


Fig. 29: *In vitro* schizontocidal activities of some fractions isolated from the roots of *Andrographis paniculata*

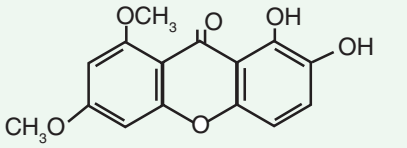
Silica gel column chromatography of fraction AG-3 eluted with chloroform resulted three distinct colour bands—yellow, greenish-yellow and pale-yellow. Two compounds with Rf values 0.7 (TDR 13008) and 0.45 (TDR 13009) were isolated from yellow coloured band by preparative TLC using benzene as mobile phase. TLC of greenish-yellow band gave four distinct spots with benzene-methanol (98:2, v/v). Compounds with Rf values 0.52 (TDR 13013) and Rf O (TDR 13011) were isolated by preparative TLC. Pale-yellow coloured band resulted four distinct spots and compound with Rf value 0.30 (TDR 130012) was isolated using benzene-methanol (95:5, v/v) by preparative TLC. Out of six compounds isolated by preparative TLC, the structure of four were determined by spectroscopic methods (Fig. 30).



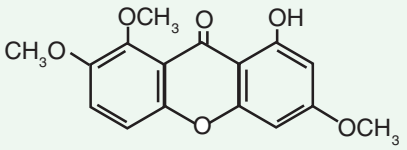
1,8-dihydroxy 3,7-dimethoxy-xanthene-9-one (TDR 13008)



4,8-dihydroxy-2,7-dimethoxy-xanthene-9-one (TDR 13009)



1,2-dihydroxy-6-8-dimethoxy- xanthene-9-one (TDR130011)



3,7,8-trimethoxy-1-hydroxy-xanthene-9-one (TDR 130013)

Fig. 30: Structures of compounds isolated from the roots of *Andrographis paniculata*

In vitro antimalarial studies showed compound TDR 13011 with maximum schizontocidal activity as compared to other compounds. However, it has exhibited moderate activity with IC50 value of 4 µg/ml-1 which has been much lower than chloroquine. Compound TDR 13011 was further assessed for its antimalarial properties *in vivo* against *P. berghei* infected mice. Results revealed that compound TDR 13011 gave substantial reduction (70%) in parasitaemia after treating animals with an intravenous dose of 30 mg/kg. Cytotoxic activity was done by WHO on MRC–5 (human lung fibroblast) showed the compound TDR 13008 to be cytotoxic with IC_{50} value 24 µg/ml-1 while all other compounds had IC_{50} values >32 µg/ml-1, indicating non-cytotoxic behaviour of TDR 13008. Our study clearly revealed that 1,2-dihydroxy-6,8 dimethoxy, xanthene-9-one isolated from the roots of *Andrographis paniculata* possessed antimalarial activity without cytotoxicity (Dua *et al* 1999).

Azadirachta indica A. Juss

Azadirachta indica A. Juss (neem) is known for its medicinal and insecticidal properties. Eight fractions from *Azadirachta indica* seeds were isolated using solvent partition and column chromatography and tested their antimalarial activity against *P. falciparum* in *in vitro* culture. Out of three fractions from seed cake, two fractions, code A-1 and A-2 showed significant activity with IC_{50} values of 4.8 and 5.0 µg/ml respectively. Similarly out of five fractions from *Azadirachta indica* oil, two fractions, code A-5 and A-6 had high antimalarial activities with their IC_{50} values of 2.25 and 2.30 µg/ml respectively while fraction code A-8 showed no antimalarial activity (Fig. 31).

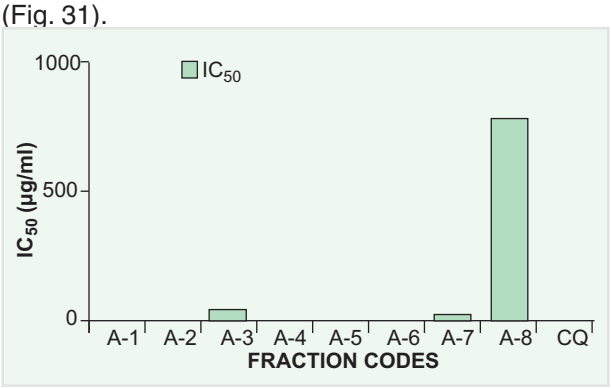


Fig. 31: *In vitro* antiplasmodial activity of *Azadirachta indica* fractions

Isolation and Testing Antimalarial Activity of Peroxydisulfate Oxidation Products of Primaquine

Primaquine, an 8-aminoquinoline, is the clinical drug of choice for the radical cure of relapsing malaria. However, its usefulness has been restricted by toxic side-effects, especially with patients deficient in glucose-6-phosphate dehydrogenase. We have isolated five compounds formed by the peroxydisulfate oxidation of primaquine using chromatographic methods and tested for antimalarial activity. *In vitro* gametocytocidal studies showed that two compounds have more gametocytocidal activity than primaquine, while *in vivo* results indicated only one compound with gametocytocidal activity against *P. yoelii* infected mice (Dua *et al* 2002).

Primaquine, on oxidation with peroxydisulfate ion in neutral medium gave pale-yellow to orange, violet and then yellow colour within one hour after initiation of reaction. Five compounds were isolated in >90% purity using Bio-Gel P-2 column chromatography and

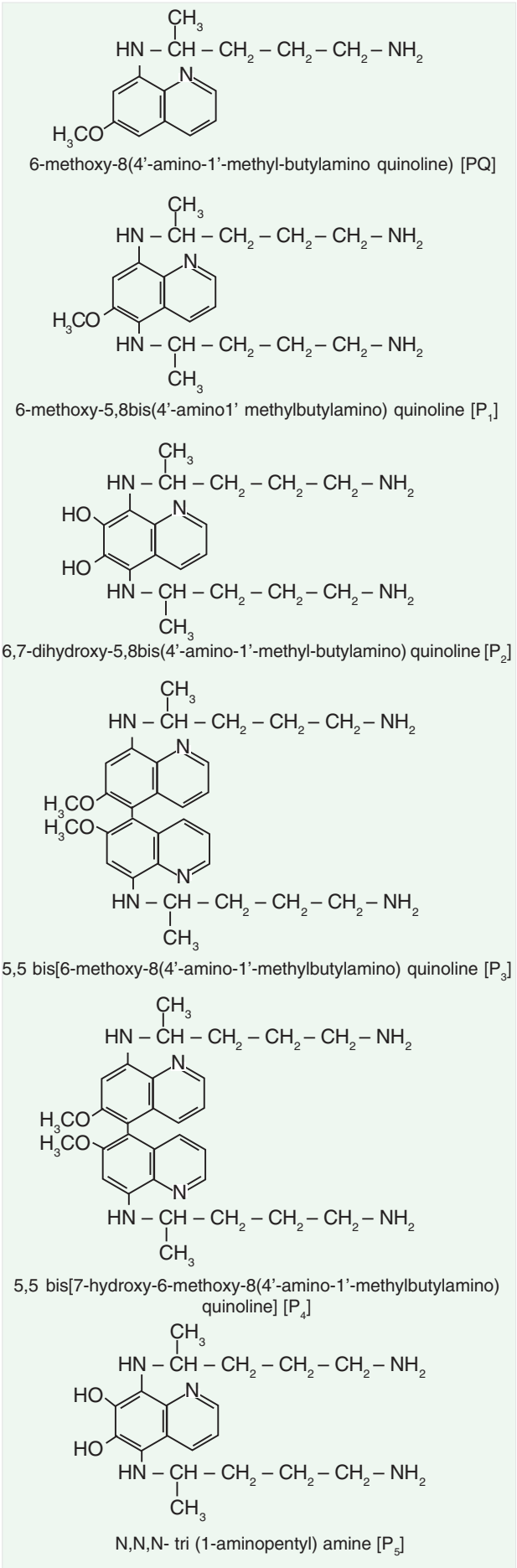


Fig. 32: Structure of oxidation products of primaquine

HPLC from the reaction mixture. The structures of all compounds were determined using IR, MS and 1H NMR studies which are given in Fig. 32.

In vitro

Five compounds isolated from the oxidation of primaquine were tested for their *in vitro* schizontocidal and gametocytocidal activities at different concentrations. Compounds P_1 and P_2 showed higher gametocytocidal activity than primaquine, while the compounds P_3, P_4 and P_5 had lower activity than primaquine or no gametocytocidal effects. The IC_{50} and IC_{90} of compound P_1 were 0.026 and 0.055 mg/well respectively, while of compound P_2 were 0.036 and 0.062 mg/well respectively. The schizontocidal activity of all five compounds were many fold lower than that of chloroquine. However, the schizontocytocidal activity of compounds P_1 and P_2 were more than primaquine.

In vivo

The compounds P_1 and P_2 were tested for *in vivo* gametocytocidal activity against *P. yoelii* infected mice. Compound P_1 showed good gametocytocidal activity in mice and there was no infectivity in mice after treatment with P_1 at the dose of 10 mg/kg. This was confirmed by feeding *An. stephensi* mosquitoes on *P. yoelii* infected mice before and after the treatment. Results showed that there was complete loss of infectivity in mosquitoes after treatment with compound P_1 while the infectivity was confirmed in mosquitoes fed on animals before treatment. Primaquine was taken as control compound. The compound P_2 did not possess any gametocytocidal effect against *P. yoelii* infected mice. In conclusion, compound P_1 [6-methoxy-5,8bis(4'-amino-1'-methylbutylamino) quinoline] is found to be a novel antimalarial compound with good gametocytocidal activity (Du*a et al* 2002).

Antimalarial Properties of Some Plants from Garhwal Region of North-west Himalaya

Studies were aimed to investigate the antimalarial properties of some plants from Garhwal region. Three plants were selected in consultation with Botanical Survey of India, Dehradun. Twelve fractions were isolated from these plants using solvent partition method. *In vitro* study was carried out on these fractions to investigate antiplasmodial activity and results are given in Table 14. Fractions isolated from plant code MRCHAR/04/3 possessed good anti-plasmodial activity while other two plants did not. Chromatographic methods are being developed for the isolation of pure compounds from plant code MRCHAR/04/3.

Antimalarial Properties of a Plant Code MRCHAR/03/04

Five compounds, coded as MRCHAR/03/04/1, MRCHAR/03/04/2, MRCHAR/03/04/3, MRCHAR/03/

Table 14. Antiplasmodium activity of some plant extracts from Garhwal region of North-west Himalaya

Fraction code	*P. falciparum* K1 IC_{50} µg/ml
MRC HAR/04/1/1	>5
MRC HAR/04/1/2	>5
MRC HAR/04/1/3	>5
MRC HAR/04/1/4	>5
MRC HAR/04/2/1	4.07
MRC HAR/04/2/2	3.7
MRC HAR/04/2/3	>5
MRC HAR/04/2/4	>5
MRC HAR/04/3/1	>5
MRC HAR/04/3/2	>5
MRC HAR/04/3/3	>5
MRC HAR/04/3/4	>5
Chloroquine	0.036

04/4 and MRCHAR/03/04/5 were tested for their antiplasmodial activity by *in vitro* method. Results revealed that fraction codes, MRCHAR/03/04/1 and MRCHAR/03/04/4 showed good activity with their IC_{50} values of 0.62 and 1.5 µg/ml respectively.

Apasmomycin Analogues

The antimalarial activity of 2-methylene-3-hydroxyalkyl (synthesized at IIT, Mumbai) propionic acid derivatives were evaluated and all of them displayed activity at 10–6 dose level in *in vitro P. falciparum* culture. Two compounds showed 100% schizont maturation inhibition at dose of 5 and 10 µmol/well respectively. *In vivo* studies of these derivatives in mice revealed antimalarial activity at 80 mg/kg dose level (Kundu *et al* 1999). Twelve t-butylperoxyamines were also synthesized at IIT, Mumbai and screened at NIMR. *In vivo* studies showed activity of one of derivatives at 80–160 mg/kg dose level (Sunder *et al* 2001). These synthetic compounds developed at IIT, Mumbai were screened *in vitro* and *in vivo* models and various levels of antimalarial activity were obtained (Kundu *et al* 1999; Sunder *et al* 2001).

Reversal of Chloroquine Resistance

Chloroquine has been the most effective and widely used drug in malaria therapy. Therefore, great hopes have been placed on development of agents, which can reverse resistance to chloroquine. Few such drugs, namely verapamil, cyproheptadine, ascorbic acid and few new compounds were evaluated *in vivo* in mice in combination with chloroquine using chloroquine resistant *P. berghei.* The results showed that these agents reversed resistance in animal models partially and that too when used in high doses, which may limit the clinical use of such systemically acting drugs (Valecha *et al* 1992, 1994). ❑