ANTIMALARIAL PROPERTIES OF SOME PLANTS FROM GARHWAL REGION OF NORTH-WEST HIMALAYA

Malaria is one of the biggest killers in the world. Current estimates place the clinical caseload almost 1 Mio deaths per year (mainly small children in Africa) and 300-400 Mio infections annually (WHO, 2010). In 2008, 109 countries were endemic for malaria, 45 of which are in Africa [World malaria report (WHO), 2008]. There were an estimated 247 million malaria episodes in 2006.

Nineteen plants were investigated belonged to Garhwal region of North West Himalaya for their antimalarial property. The results from in vitro antiplasmodial screening from both schizont maturation and pLdh methods revealed that fractions from *A. vasica, A. roxburghiana, H. antidysentrica, C. bonducella, S. ciliata, R. cinerea, A. bracteosa, L. cephalotes, M. piperita* and *V. canescense* possessed antiplasmodial activity with their IC_{50} values≤ 15 µg/ml while other fractions have their IC_{50} values >15 µg/ml thus found in-actives. 10 out of 80 extracts were most active against *P. falciparum* K1 strain, while remaining extracts were considered as non active. Chloroform extracts of *A. vasica* (AV-2), *A. bracteosa*(AB-2), *M. piperita*(MP-2), and *S. ciliata*(SC-2) showed very low antiplasmodial activity. While chloroform extract of *H. antidysentrica* (HA-2) and dichloromethane extract of *C. bonducella*(CB-2) showed moderate antiplasmodial activity. Similarly petroleum ether extract from *A. roxburghiana*(AR-1) and *R. cinerea*(RC-1) exhibited good antiplasmodial activity while chloroform extract of *A. roxburghiana*(AR-2), *R. cinerea*(RC-2) and *L. cephalotes*(LC-2) also showed good antiplasmodial activity. Only the chloroform extract from *A. roxburghiana* (AR-2) exhibited very good antiplasmodial activity with its IC_{50} value 0.417 µg/ml. Other plant extracts exhibited no inhibition against *P. falciparum* isolate.

The cytotoxicity assays of all extracts possessed IC_{50} values > 16 µg/ml and were not cytotoxic (as per WHO criteria) except petroleum ether extract of plant *V. canescense* (Violaceae) with IC_{50} value 12.39 µg/ml. The selectivity index (SI) of these active plant extracts was also determined. The chloroform extract of plant *A. roxburghiana, R. cinerea* and *L. cephalotes* exhibited 8 to 78 fold activity against *P. falciparum* than against the rat L-6 cell line. The petroleum ether extract of *A. roxburghiana, V. canescens* and, *R. cinerea* showed 4 to 10 fold activity against *P. falciparum* than against the rat L-6 cell line.

6 out of 80 extracts were found most active against parasites tested beside *P. falciparum isolate*, remaining extracts were considered as non active against these parasites. 2 extracts were effective against amastigotes *of L. donovani*, one extract was found effective against amastigotes *of T. cruzi* and 3 extracts were effective against trypomastigotes of *T. b. rhodesiense*.

Extract showed promising results were fraction code AR-1, AR-2 from *A. roxburghiana*; CB-2 from *C. bonducella*; HA-2 from *H. antidysentrica*; LC-2 from *L. cephalotes*; RC-1, RC-2 from R. cinerea and VC-1 from *V. canescense*. These extract were assessed for its antimalarial properties in in-vivo system against plasmodium berghei infected mice. Results revealed from in vivo studies of these fractions that fraction code AR-2 from *A. roxburghiana* had substantial antimalarial property, gave substantial reduction 58.8 % in parasitemia after treating the animal with an intraperitonial dose of 30 mg kg^{-1} while fraction code AR-1 did not possess any activity. Similarly fraction code RC-2 had substantial antimalarial property; reduce substantial

parasitemia 60.3 % after treating the animal with an intraperitonial dose of 30 mg kg^{-1} while fraction code RC-1 did not have any activity. Fraction code CB-2 from *C. bonducella* showed antimalarial property with 59.33% reduction in parasitemia after treating the animal with an intraperitonial dose of 30 mg kg^{-1}. Similarly fraction code HA-2 from *H. antidysentrica* demonstrate 70.2% reduction in parasitemia after treating the mice with an intraperitonial dose of 30 mg kg^{-1}. While fraction code LC-2 from *L. cephalotes* did not quite reach the level of chemosupression seen with the drug used as positive control chloroquine. Due to potent inhibition of VC-1 against *P. falciparum* isolate, it is evaluated for *in-vivo* antiplasmodial activity against *P. berghei* infected mice. The percent reduction of parasitemia of VC-1 extract was found 63% on 20 mg/kg dose. Results from *in-vivo* antimalarial activity revealed that VC-1 showed significant reduction of parasitemia on dose ranges 10-20 mg/kg, without any mortality in animals of the of VC-1 extract treated group as compared to the animals of control group. Whereas the dose of 30 mg/kg showed mortality and weight loss in the animals of VC-1 extract treated group, indicates the margin of safety of the drug might be less. It is unknown whether the inhibition by VC-1 extract is caused by specific antiplasmodial action or general toxicity.

Five out of nineteen plants namely *A. roxburghiana*; *C. bonducella; H. antidysentrica*; *L. cephalotes*; *R. cinerea* and *V. canescense* showed antimalarial activity in crude form, Once it was established from in vitro and in vivo studies that essentially compounds were responsible for the antimalarial activity of these extracts, therefore targeted isolation of the active compounds from these extracts was conducted using different chromatographic methods and their characterization was done using spectroscopic methods followed by antimalarial screening.

The bioassay-guided fractions were isolated from the active plant using different chromatographic methods (column, thin layer chromatography, high performance liquid chromatography). These isolated pure compounds were than asssed for their antimalarial activity (In vitro and in vivo) and finally characterize and identified by mass spectrometry (MS), fourier transform Infrared spectroscopy (FTIR) and NMR experiments. Compounds responsible for antimalarial activity of *A. roxburghiana* are fiedelan-3-one with 89% reduction in parasitaemia and quercetin-3;3',4'-trimethyl ether with 56.5% reduction in parasitaemia. Similarly compounds responsible for antimalarial activity of *C. bonducella* were norcaesalpinin A and norcaesalpinin B. Results revealed that compound Norcaesalpinin A gave substantial reduction (68.81%) in parasitaemia after treating the animals with an intraperitonial dose of 30 mg kg−1 and compound Norcaesalpinin B gave 60.83% reduction in parasitaemia after treating the animals with an intraperitonial dose of 30 mg kg−1. While On the basis of spectroscopic methods the fractions from *H. antidysentrica* were identificatied as conessine and holadysenterine. These compounds were than assessed for its in vivo antimalarial activity against *P. berghei* infected Swiss albino mice using Peter's 4 days test. Results revealed that compounds gave substantial reduction 73.25% and 42.03% in parasitaemia after treating the animals with an intraperitonial dose of 30 mg kg−1.

Many studies related to ethnopharmacology aim to correlate pharmacological activities detected in a traditional remedy with chemical active constituents, through natural product chemistry techniques. Therefore ethnopharmacology, interdisciplinary in its nature, tries to combine two points of view, a cultural and biological one, in a complementary approach. Ethnopharmacology has gained recognition at both scientific and popular levels. However there is a lack of systematic knowledge in the field of malaria. The main goal here is not only to detect a new future ''ethno-lead'', but primarily to produce economically affordable plant-based antimalarial products. Is it a sign of the return of an interest in medicinal plants. At least it presents a new challenge to those

interested in finding cures, whether old or new. Our study could be helpful to formulate a potent herbal product with good antimalarial activity, which is the need of time against malaria.