Abstract

Malaria, the lethal disease is caused by the protozoan parasite of the genus *Plasmodium* which is transmitted through the bites of female *Anopheles* mosquitoes. Among the five species that infect humans, *P. falciparum* is considered as the most virulent species and is responsible for most of the malaria-related deaths. The virulence of the species is attributable to the presence of an astonishing array of sequences and genes that play key roles in pathogenesis and immune evasion. Diagnosis and treatment of the disease are the most essential interventions in addition to the preventive measures that include vector control strategies. The major challenges to the treatment intervention are posed by the complex lifecycle of the parasite along with its inherent ability to evolve through mutation and sexual recombination in response to drugs, providing a moving target. After the failure of chloroquine and sulfadoxine-pyrimethamine in some parts of the world, at present, the artemisinin-based combination therapies remain the most effective method to treat the resistant parasite but again with the reports of multidrug resistance. Thus, there is an urgent need to discover new antimalarial drugs. Hemoglobin degradation is a metabolic process essential for the survival of the *Plasmodium* parasite, and in *P. falciparum* the process is initiated by the two aspartic proteases in the parasite's food vacuole namely plasmepsin I (PM I) and plasmepsin II (PM II). Therefore, the enzymes are strongly suggested as the potential targets for novel antiplasmodials. Plants contain myriads of molecules with diverse pharmacological properties, indeed many of the molecules are yet to be discovered. Central to the thesis is finding the antiplasmodial agents from plants that act by inhibiting the *P. falciparum* enzymes PM I and PM II.

Initially, 16 plants were selected based on the ethnopharmacological approach. The extracts prepared from different parts of the plants were screened for the antiplasmodial activity against *P. falciparum* 3D7 *in vitro*. Out of the 129 extracts tested, 22 exhibited significant antiplasmodial activity. These antiplasmodial extracts were then investigated for their ability to inhibit mPM I and mPM II, the recombinantly expressed *P. falciparum* 3D7 PM I and PM II respectively. Of the 22 extracts studied 4 extracts namely, the *A. paniculata* EtOH (aerial parts), *C. wightii* AQ (stem), *C. zedoaria* DCM (rhizome) and *P. amarus* DCM (aerial parts) extracts inhibited both the enzymes significantly when tested *in vitro*. However, after *in vitro* cytotoxic activity analysis performed using HEK-293 cells and *in vitro* hemolytic

activity analysis done using human erythrocytes, the *A. paniculata* EtOH and *C. wightii* AQ extracts were concluded as safe and these extracts were selected for further studies.

PM I and PM II were recombinantly expressed from *P. falciparum* 3D7 in *E. coli* BL21(DE3)pLysS expression system to meet the requirement for the inhibition studies. In the parasite, PMs are expressed as inactive zymogens, which are activated to their mature active form following the removal of the prosegment. Recombinant expression of PMs and attaining their active forms have generally been challenging. An earlier study has demonstrated that when expressed without the prosegment, PM II could achieve its active conformation without undergoing the activation process. Using a similar approach mPM I and mPM II, the enzymes PM I and PM II with only their mature segments were expressed. Following purification, the enzymes were refolded using the thermal-assisted refolding technique into their active forms which were capable of degrading their natural substrate, hemoglobin. The current work presents a convenient method to refold PMs into their active forms.

Phytochemical analysis of the two extracts with PM inhibition activity was done. Several compounds from the *A. paniculata* EtOH extract were identified along with the two principal compounds andrographolide and 14-deoxy-11, 12-didehydroandrographolide. Interestingly the over-abundance of different classes of phospholipids was also revealed in the extract, which possibly arises a new research question. The powerful antioxidant scroside-D or a compound alike, was found as one of the major compounds identified from the *C. wightii* AQ extract. The extract was found to be abundant in free amino acids and saponins.

Molecular interaction studies between the compounds identified from both the extracts and the enzymes mPM I and mPM II were performed to identify the potential inhibitors from the extracts and to gain insights into their possible mode of action. Four compounds viz., 2-amino-2-ethyl-4-(methylsulfonimidoyl)butanoic acid and andrographolide from the *A. paniculata* EtOH extract and safrole and sorbitol hexaacetate from the *C. wightii* AQ extract were found to interact with the catalytic dyads of both the enzymes, which marks them as very important PM blocking agents. The compounds 2-amino-2-ethyl-4-(methylsulfonimidoyl)butanoic acid and andrographolide which formed more stable complexes with the enzymes can be taken as lead structures to develop new antiplasmodial drugs that target PM I and PM II of *P. falciparum.*