Summary

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Chapter 2: Screening of medicinal plants against *Plasmodium falciparum*

- Based on ethnopharmacological approach 16 plants were selected for the determination of antiplasmodial potential. Among all the 129 extracts derived from the plants, 22 extracts showed > 70% inhibition of the *P. falciparum* 3D7 parasite *in vitro*, which were selected for further studies.
- Out of all the 22 antiplasmodial extracts tested *in vitro* for their ability to inhibit the recombinantly expressed parasite's food vacuole enzymes, mPM I and mPM II, four extracts viz. *A. paniculata* EtOH, *C. wightii* AQ, *C. zedoaria* DCM and *P. amarus* DCM extracts exhibited > 50% inhibition of both the enzymes. All the 22 extracts tested were found to inhibit both the enzymes almost equally and a positive correlation was found between the extent of the inhibition of both the enzymes by the extracts, which were expected outcomes due to the similarity in the active site of the enzymes.
- From the *in vitro* cytotoxicity analysis of the four extracts with PM inhibition activity the *A. paniculata* EtOH and *C. wightii* AQ extracts were found to be non-toxic whereas the *C. zedoaria* DCM and *P. amarus* DCM extracts were found to be cytotoxic against HEK-293 cells.
- The four extracts with PM inhibition activity when studied *in vitro* for their hemolytic effect, did not display any significant hemolytic activity against human erythrocytes till the concentration of 250 µg/ml, hence were concluded to be non-hemolytic.
- Overall the *A. paniculata* EtOH and *C. wightii* AQ extracts were found to be highly selective for the parasite and taken forward for further studies.

Chapter3: Cloning and expression of Plasmepsin I and Plasmepsin II from *Plasmodium falciparum* and search for their inhibitors

- The full-length genes for PM I and PM II consisting of 1359 bp and 1362 bp respectively were amplified from *P. falciparum* 3D7. Further, the full-length genes were used as templates to obtain 1008 bp regions harboring the mature part of the enzymes mPM I and mPM II respectively.
- The fragments carrying mPM I and mPM II regions were cloned into pET-28a(+) vector that resulted in mPM I and mPM II constructs.

- The constructs mPM I and mPM II were transformed in the *E. coli* DH5α cloning host and the amplified constructs isolated from the transformants were thereafter used to transform the expression host *E. coli* BL21(DE3)PLysS. One transformant each for the constructs mPM I and mPM II were selected for further studies and they were named mPM I PLysS and mPM II PLysS respectively.
- Upon induction mPM I PLysS and mPM II PLysS expressed the His-tag fusion enzymes, mPM I and mPM II of less than 43 kDa, as expected from their theoretically determined molecular weights of 39.51 kDa and 39.37 kDa respectively.
- Following the purification pipeline, the pure enzymes mPM I and mPM II without His-tag of 37.63 kDa and 37.49 kDa respectively were achieved.
- Using the thermal-assisted refolding technique the His-tagged removed enzymes were successfully converted to their active forms which were capable of degrading their natural substrate hemoglobin.
- The yields of the final purified active forms of mPM I and mPM II enzymes were found to be 110 µg/l culture and 120 µg/l culture respectively.

Chapter 4: Phytochemical characterization of *Andrographis paniculata* ethanol and *Commiphora wightii* aqueous extracts

- The EtOH extract of *A. paniculata* and AQ extract of *C. wightii* with PM inhibition property were subjected to phytochemical analysis. The major phytochemicals found in the *A. paniculata* EtOH extract were alkaloids, carbohydrates, phenolics, proteins, steroids and terpenoids while in the case of *C. wightii* AQ extract alkaloids, amino acids, carbohydrates, phenolics and saponins were found as the major classes of phytochemicals.
- The LC-MS-QTOF analysis of the *A. paniculata* EtOH extract led to the identification principle components of the extract that included 14-deoxy-11, 12-didehydroandrographolide and andrographolide which belong to the class of terpenoids, that constituted almost 50% of the extract as indicated by the quantitative phytochemical analysis.
- The LC-MS-QTOF analysis also revealed the unexpected over-abundance of phospholipids in the *A. paniculata* EtOH extract. Explaining this profusion of phospholipids in the plant presents a new research question.

- The LC-MS-QTOF analysis of *C. wightii* AQ identified the seven principle compounds of the extract along with a compound similar to Scroside D. Large amounts of C_{35}-C_{39} fragments which are suggested to be derivatives of compounds belonging to the class saponins were also found after the analysis.
- The abundance of saponins and the absence of hemolytic activity found in the case of *C. wightii* AQ extract corroborates the statement that various structural aspects, such as the nature of the aglycone backbone, the complexity of sugar moieties and the number, length and position of sugar side chains, may affect hemolytic activity.

Chapter 5: Molecular interaction studies of *Plasmodium falciparum* specific Plasmepsin-I and Plasmepsin II with compounds from *Andrographis paniculata* ethanol and *Commiphora wightii* aqueous extracts

- To identify the possible inhibitor(s) of mPM I and mPM II and also to gain insights into the mode of their inhibition, the interactions between the compounds identified from *A. paniculata* EtOH and *C. wightii* AQ extracts and the enzymes mPM I and mPM II were studied *in silico*.
- The 3D structures for mPM I and mPM II were predicted by the Phyre2 Protein Fold Recognition Server using 2BJU as a template. The quality of both the structures generated was found to be high suggesting that they represented the native structures of the enzymes with high accuracy.
- Total 13 compounds from the two extracts were docked against both the enzymes mPM I and mPM II using the docking software tool AutoDock 4.2.6.
- 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 have a high binding affinity for the active site of both the enzymes.
- In the case of both mPM I and mPM II enzymes, 2-amino-2-ethyl-4-(methylsulfonimidoyl)butanoic acid and andrographolide from *A. paniculata* formed H-bond with the amino acid residues in the active site of the enzymes. Furthermore, in the case of mPM I, the former formed H-bond with the catalytic aspartic acid residue Asp40, while it formed hydrophobic contacts with the other catalytic aspartic acid residue Asp220 and all other residues in the vicinity of the catalytic dyad. Also, the former in the case of

mPM II and the later in the case of both the enzymes formed hydrophobic bonds with the catalytic dyad aspartic acid residues and the residues in the vicinity of the catalytic dyad.

- Safrole and sorbitol hexaacetate from the *C. wightii* AQ extract were found to make hydrophobic contacts with the catalytic dyad aspartic acid residues and the residues in the vicinity of the catalytic dyad of both the enzymes.
- The blocking of the active site residues is proposed as the possible mechanism of mPM I and mPM II inhibition by the four compounds which was found during the *in vitro* studies with the extracts.
- The fact that H-bonds form stable protein-ligand complex suggests 2-amino-2-ethyl-4-(methylsulfonimidoyl)butanoic acid and andrographolide as more promising inhibitors of mPM I and mPM II as compared to safrole and sorbitol hexaacetate.
- 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 are suggested as antiplasmodial agents from the plants selected using ethnopharmacological approach, that work by targeting the *P. falciparum* food vacuole enzymes PM I and PM II.