Phytochemical and Pharmacological Investigation of Four Tanzanian Medicinal Plants for Anti-Protozoal Activity

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Protozoal diseases like malaria, trypanosomiasis and leishmaniasis, caused by *Plasmodium*, *Trypanosoma* and *Leishmania*, respectively, have overwhelming impact on public health in developing regions. Due to their prevalence, virulence and drug resistance, they are the most serious and widespread parasitic diseases encountered by mankind. The inadequate armory of drugs, high cost and lack of new drugs are the major limiting factors in the fight against the three diseases. Consequently, there is continuing need of research on the discovery and development of new effective and safe anti-protozoal drugs. In this work, solvent extracts from four Tanzanian medicinal plants: *Annickia kummeriae*, *Pseudospondias microcarpa*, *Drypetes natalensis* and *Acridocarpus chloropterus*, were tested for anti-protozoal activity. Anti-protozoal bioassays revealed 14 solvent extracts with strong to moderate *in vitro* anti-plasmodial activity (IC$_{50}$ 0.12±0.01-5.50±0.27 µg/ml), 6 with moderate anti-trypanosomal activity (IC$_{50}$ 2.3±0.43-5.40±0.64 µg/ml) and only 3 with mild anti-leishmanial activity (IC$_{50}$ 9.25±0.54-9.79±2.5 µg/ml). *Annickia kummeriae*, *P. microcarpa* and *D. natalensis* solvent extracts exhibited good anti-plasmodial activity and favourable selectivity (SI 29.2-2,250). The strong anti-plasmodial, moderate anti-trypanosomal and mild anti-leishmanial activity of the methanolic extract of *A. kummeriae* leaves encouraged the isolation of anti-protozoal compounds. Bioassay-guided chromatographic fractionation led to isolation and identification of fractions with stronger anti-plasmodial activity and more favourable selectivity than the solvent extracts. Seven anti-protozoal alkaloids: palmatine (82), jatrorrhizine (83), lycicamine (127), trivalvone (128), annickine (129), columbamine (130) and (-)-tetrahydropalmatine (131), were identified by spectroscopic methods and subjected to *in vitro* anti-protozoal assays. The isolated alkaloids showed good selectivity and strong to moderate anti-plasmodial activity (IC$_{50}$ 0.08±0.001-2.3±0.44 µg/ml), mild to weak anti-trypanosomal (IC$_{50}$ 3.2±0.01-14.30±0.1 µg/ml) and anti-leishmanial activity (IC$_{50}$ 2.7±0.1-20.4±0.1 µg/ml). Chromatography of *A. chloropterus* extract led to the isolation and identification of 5 triterpenes: β-sitosterol (132), stigmasterol (133), friedelin (134), oleanolic acid (124), ursolic acid (123); and 5 flavonoids: apigenin (135), luteolin (136), vitexin (137), kaempferol (138) and quercetin...
Quercetin (139) exhibited moderate in vitro anti-plasmodial activity (IC₅₀ 2.6±0.05 µg/ml) while the rest of compounds were inactive. Mild to weak in vitro anti-trypanosomal activity was observed in quercetin (139) (IC₅₀ 3.60±0.1 µg/ml), ursolic acid (123) (IC₅₀ 7.80±0.1 µg/ml) and apigenin (135) (IC₅₀ 9.0±0.1 µg/ml). Good in vitro anti-leishmanial activity (IC₅₀ 0.80±0.001, 2.10±0.1, 2.20±0.1, 5.90±0.1 and 3.5±0.2 µg/ml) and favourable selectivity were observed with ursolic acid (123), quercetin (139), kaempferol (138), apigenin (135) and oleanolic acid (124), respectively. Chemical structure-biological activity relationship comparisons confirmed that, the methoxyl groups at C-2, C-3 (ring A) and C-9, C-10 (ring D) together with the quaternary nitrogen atom in position 7 are the structural moieties required for strong anti-plasmodial activity in protoberberine alkaloids. Structure-activity-relationship (SAR) comparisons in the isolated triterpenoids confirmed that, the hydroxyl group at C-3 with methyl groups at C-23, C-25, C-26 and C-30, C-12/C-13 double bond, the carboxylic acid group at C-28, and hydrogen at C-20 in the molecular framework of ursolic acid (123) are the structural moieties responsible for the strong anti-leishmanial activity whereas in the isolated flavonoids, the hydroxyl groups at C-3 and C-3′ in the apigenin (135) molecular framework are the structural moieties responsible for the strong anti-protozoal activity observed. The alkaloids with strong anti-plasmodial and moderate anti-trypanosomal activity may provide lead compounds for the development of drugs for malaria and trypanosomiasis. Similarly, the strong to moderate anti-leishmanial activity of the isolated triterpenes and flavonoids render them good candidates as molecular templates for new drug development.