Journal of Medicinal Plants Research Vol. 4(9), pp. 726-731, 4 May, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.188
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

Antiprotozoal activity and cytotoxicity of metabolites from leaves of *Teclea trichocarpa*

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Accepted 5 January, 2010

Chromatographic separation of the leaves of *Teclea trichocarpa* (Engl.) Engl. (Rutaceae) used traditionally by Akamba tribe in Kenya yielded three acridone alkaloids, a furoquinoline alkaloid and two triterpenoids. The total extract (methanol) of the leaves of this plant and the isolated compounds were screened for *in vitro* for cytotoxicity and against parasitic protozoa, *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani*. Among the compounds α-amyrin had the best anti-plasmodial activity (IC_{50} = 0.96 μg/ml), normelicopicine and skimmianine had the best anti-trypanosomal activity against *T. b. rhodesiense* (IC_{50} = 5.24 μg/ml) and *T. cruzi* (IC_{50} = 14.50 μg/ml), respectively. Normelicopicine also exhibited best anti-leishmanial activity (IC_{50} = 1.08 μg/ml). Arborinine exhibited moderate cytotoxicity (IC_{50} = 12.2 μg/ml) against L-6 cells. The compounds with low anti-protozoal and high cytotoxicity IC_{50} values are potential source of template drug against parasitic protozoa.

Key words: Cytotoxicity, *Teclea trichocarpa*, protozoa, alkaloids.

INTRODUCTION

Protozoal diseases are the oldest and most devastating tropical diseases affecting man and animals. They are responsible for considerable mortality throughout the world but are predominant in the tropics and sub-tropics (WHO, 2002). Malaria, trypanosomiasis and leishmaniasis are protozoal diseases caused by the parasites of the genus *Plasmodium*, *Trypanosoma* and *Leishmania*, respectively. They exhibit a combination of biochemical, physiological, nutritional adaptation and have a tendency to induce immunity in their hosts that allow them to survive (Olliaro et al., 1996).

Malaria is the most serious and wide spread parasitic disease encountered by mankind. Each year, about 500 million people are afflicted and about 2.7 million people five years old and pregnant women due to lack of or low immunity (WHO, 2002). Resistance of malarial parasites to commonly used anti-malarial drugs such as chloroquine, halofantrine and sulfadoxine-pyrimethamine (Kain, 1995) has highly demanded the search for effective, safe and affordable drugs to be one of the pressing health priorities.

Trypanosomiasis is another highly disabling and fatal disease, common in Africa and Latin America. The Africa trypanosomiasis (sleeping sickness) is found only in Africa where about 50,000 new cases are reported annually (Molyneux et al., 1996). Its treatment is still a challenge due to marginal efficacy and severe toxicity of available drugs, therefore, a need for search of new less toxic and more effective drugs (Wright and Phillipson,

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1990). Leishmaniasis afflicts large areas of Africa, Asia, Mediterranean and Latin America. It is transmitted to humans through bites by sand flies (Diptera, Psychodidae). The disease has wide spectrum of clinical diseases ranging from destructive and disfiguring sores to *kala azar*, which destroys internal organs (Bryceson, 1996). Treatment of leishmaniasis is also inadequate and is severely limited due to marginal efficacy, adverse and toxic side effects of the currently available drugs (Wright and Phillipson, 1990). The control of vectors of the parasites based on application of residual insecticides and minimizing vector-human contact is increasingly becoming difficult due to resistance developed against available commercial insecticides (WHO, 1984). Secondly, it is important to note that only a limited number of insecticides are available for use in public health.

Immunoprophylaxis is yet to bear appreciable results in the control of parasitic protozoan diseases. These protozoan parasites exhibit a number of different developmental stages in their life cycle hence candidate vaccines are based on various antigens derived from these stages. The development of effective vaccine has been plagued with a number of shortcomings, many of which are related to the antigenic variations and diversity coupled with immune evasion mechanisms exhibited in various developmental stages (Howard, 1987).

Herbal remedies have been considered to be important source for lead compounds or as phytomedicine due to their use in the treatment of different ailments since antiquity. However, only about 20% of the plants with claimed bioactivities have been subjected to bioassay screening (Houghton, 2001). In Kenya, plant extracts are still widely used in treatment of various ailments. Many plants derived natural products have been reported to exhibit antiprotozoal activities against parasitic protozoa (Wright and Phillipson, 1990).

Teclea trichocarpa (Engl.) Engl. is used by traditional healers belonging to the Akamba tribe of East Africa for malaria treatment, as anthelmintic and inhale the vapour as a cure for fever (Watt and Breyer-Brandwijk, 1962). Some other species of *Teclea* are also used in Africa including *T. nobilis* the bark and leaves are used as analgesics in Ethiopia (Mascolo et al., 1988). *T. ouabanguiensis* is used as a remedy for coughs and asthma in Cameroon (Watt and Breyer-Brandwijk, 1962).

Previous biological studies of the plant revealed potent insect antifeedant activity against the African armyworm, *Spodoptera exempta*, antifungal and antibacterial activity (Lwande at al., 1983) and *in vitro* antiplasmodial activities (Muriithi et al., 2002). Previous phytochemical studies led to isolation of melicopicine, 6-methoxytecleanthine tecleanthine, normelicopicine, arborinine, skimmianine and dictamine (Lwande et al., 1983; Muriithi et al., 2002).

In order to verify the efficacy and safety of folk medicines this plant was studied for the *in vitro* cytotoxicity and against parasitic protozoa.

Thus, in this paper we report the antiplasmodial, anti-trypanosomal, anti-leishmanial and cytotoxicity activities of the total organic crude extract and six compounds isolated from *T. trichocarpa*.

MATERIALS AND METHODS

Materials and chemicals

The leaves of *T. trichocarpa* were collected from Chesoi, Marakwet District, Rift Valley Province, Kenya in August, 2007. Plant authentication was done by plant taxonomist, Mr. L.K. Karimi, Department of Pharmacy, Kenyatta University, and the Voucher specimen (SM/TT/002/2007) was deposited in the Department of Botany Herbarium at Kenyatta University, Nairobi. Melting points were determined on an electro thermal melting point apparatus and expressed in degree centigrade and are uncorrected. IR spectra were taken in KBr pellets and recorded on a Shimazdu (model FT-IR-8400 CE) with absorption given in wave numbers (cm^{-1}). NMR spectra were recorded at room temperature on a Bruker DPX-400 NMR. The spectra were recorded in $CDCl_3$ as the solvent and TMS as the internal standard. The chemical shifts were reported in δ (ppm) units relative to TMS signal and coupling constant (J) in Hz. Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel 60 F_{254} (Merck) with a 0.2 mm layer thickness. Preparative TLC was done using normal phase silica gel (F_{254} Merck) pre-coated on aluminium plate (20 × 20 cm) and a layer thickness of 0.25 mm. Spots on chromatograms were examined under UV light (λ 254 and 366 nm), using a hand lamp (Model UV GL-58 mineral light lamp of 254 - 366 nm) and sprayed with an anisaldehyde and Dragendorff's visualization reagents. VLC column were packed with thin layer chromatography silica gel 60 (6 - 35 microns mesh, ASTM) and column chromatography silica gel 60 (0.040 - 0.063 mm, 230 - 400 mesh, Merck).

Extraction, fractionation and isolation

The air-dried leaves (2.0 kg) of *T. trichocarpa* were ground to a fine powder using a blender and sequentially extracted (×3) with *n*-hexane, dichloromethane and methanol at room temperature, in addition, from the ground powdered material total organic extract was obtained. Each extract was concentrated *in vacuo* at 40°C. Extraction yielded 26.0 g of hexane, 52.4 g of dichloromethane and 199.2 g of methanol extracts respectively. When CH_2Cl_2 extract was subjected to VLC (TLC silica gel) and CC on silica gel, followed by preparative TLC eluting with hexane and CH_2Cl_2 yielded four alkaloids; melicopicine (1) (38.1 mg), normelicopicine (2) (46.9 mg), arborinine (3) (99.0 mg) and skimmianine (4) (22.2 mg). When the *n*-hexane extract was subjected to similar chromatographic separation, afforded a common plant sterol β-sitosterol (5) (43.1 mg) and the less common α-amyrin (6) (32.7 mg). The extract obtained by extracting powdered leaves (100 g) in methanol (3 × 500 ml) for 24 h concentrated *in vacuo* at 40°C and the methanol extract thus obtained was used in the antiprotozoal and cytotoxicity assay.

Biological activity tests

The *in-vitro* parasitic protozoa and cytotoxicity assays were carried out at the Swiss Tropical Institute (STI), Basel. Table 1 lists the parasites, strain(s), stage of the parasites and the standard used for each parasite.

Table 1. Parasites *in vitro* assays.

Parasite	Strain	Stage	Standard
Plasmodium falciparum	K1	IEF	Chloroquine
Trypanosoma brucei rhodesiense	STIB 900	Trypomastigotes	Melarsoprol
Trypanosoma cruzi	Tulahuen C4	Amastigotes	Benznidazole
Leishmania donovani	MHOM-ET-67/L82	Amastigotes	Miltefosine

***Plasmodium falciparum in vitro* assay**

The anti-malarial assay was carried out to evaluate the activity of the pure compounds against *Plasmodium falciparum* (K1 strain, chloroquine resistance). *P. falciparum* were maintained *in vitro* on human red blood cells by preventing the uptake of radio-labeled nucleic acid precursor [^{3}H]-hypoxanthine (Ridley et al., 1996). Infected human red blood cells were exposed to serial dilutions in micro-titre plates for 48 h and chloroquine (Sigma C6628) were used as the standard drug. Viability was assessed by measuring the incorporation of [^{3}H]-hypoxanthine by liquid scintillation counting. From the sigmoidal inhibition curves IC_{50} values were calculated (Matile and Pink, 1990).

Trypanosome *in vitro* assay (Alamar blue assay)

The *in vitro* anti-trypanosoma assay was carried out to evaluate the ability of the crude and pure compounds to inhibit growth of *Trypanosoma brucei rhodesiense* (STIB 900) and *Trypanosoma cruzi* (Tulahuen C4). Serial drug dilutions in minimum essential medium supplement were added to microtitre plates. Trypomastigotes of *T. b. rhodesiense* were added to each well and incubated for 72 h. Viability was assessed by Alamar Blue leading to a colour reaction, which was read in a fluorescence scanner (Millipore Cytofluor 2300) (Räz et al., 1997). Fluorescence development was expressed as percentage of the control and IC_{50} values determined.

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtitre plates, after 24 h trypomastigotes of *T. cruzi* were added to the wells and plates incubated at 37°C in 5% CO_2 for 4 days. The substrate CPRG/ Nonidet was added to the wells for measurement of the IC_{50}. The colour reaction, which developed during the following 24 h, was read photometrically at 540 nm and from the sigmoidal inhibition curve, IC_{50} values were calculated.

Leishmanicidal assay

Mouse peritoneal macrophages were seeded in RPMI 1640 medium with 10% heat inactivated FBS in Lab-tek 16-chamber slides. The *Leishmania donovani* (MHOM/ET/67/L82) amastigote were added at a ratio of 3: 1 (amastigotes: macrophages). The medium containing free amastigotes was replaced by fresh medium 4 h later. Next day the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37°C under a 5% CO_2 atmosphere for 96 h, and then the medium was removed, the slides fixed with methanol and stained with Giemsa. The ratio of infected to non-infected macrophages was determined microscopically, expressed as percentage of the control and the IC_{50} values calculated by linear regression (Cunningham, 1977).

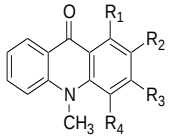
Cytotoxicity assay

The i*n vitro* cytotoxicity test was evaluated by use of macrophage and L-6 cells and podophyllotoxin as the standard. Pure compounds were dissolved in 0.5% dimethylsulphoxide (DMSO) and serial dilutions were prepared by using a 12-channel multipipette. The IC_{50} value was determined by adding 10 μl of the fluorescent dye Alamar blue (Trek Diagnostic East Grinstead, UK) to each well and plates incubated for 2 h. The plate was read using fluorescence plate reader. The data obtained was transferred into a graphic program (Excel) and sigmoid curves generated from which IC_{50} values were calculated and expressed as minimum inhibitory concentration (MIC) in μg/ml (Matile and Pink, 1990).

RESULTS AND DISCUSSION

The percentage yields of hexane extract (1.3%) were the lowest, with twice and eight fold percentage yield of CH_2Cl_2 (2.6%) and MeOH (10%) extracts, respectively. The TLC profile of *T. trichocarpa* extracts revealed the presence of several UV active and fluorescing compounds in the crude extracts. Chromatographic separation of the hexane and CH_2Cl_2 extracts afforded three acridone alkaloids, melicopicine (1), normelicopicine (2) and arborinine (3), a furoquinoline alkaloid, skimmianine (4) and two triterpenoids, β-sitosterol (5) and α-amyrin (6). The percentage yields of isolated compounds are listed in Table 2.

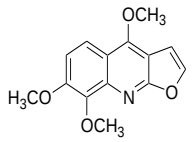
The structures of the compounds were characterized and identified by their IR, ^{1}H and ^{13}C NMR and comparing with data of authentic samples (Bergenthal et al., 1979; Lwande et al., 1983; Mahato and Kundu, 1994; Rasoanaivo et al., 1999; Muriithi et al., 2002).



(1) Melicopicine (R_1, R_2, R_3, R_4 = OCH_3

(2) Normelicopicine (R_1= OH, R_2, R_3, R_4 = OCH_3)

(3) Arborinine (R_1 = OH, R_2, R_2, R_3 = OCH_3, R_4 = H)



(4) Skimmianine

The total (methanolic) crude extract and the six compounds isolated from *T. trichocarpa* were tested *in vitro* against parasitic protozoa; *P. falciparum*, *T. b. rhodesiense*, *T. cruzi* and *L. donovani*. Results are summarized in Table 3. The values are expressed in inhibitory doses (IC_{50}) in μg/ml.

Anti-plasmodial activities of the crude extracts and isolated compounds together with that of control drug

Table 2. Extracts with percentage yield of compounds.

Compound	Extract	Percentage yields (%)
β–Sitosterol (5)	Hexane	0.166
α-Amyrin (6)		0.126
Melicopicine (1)	CH_2Cl_2	0.072
Normelicopicine (2)		0.089
Arborinine (3)		0.189
Skimmianine (4)		0.042

Table 3. *In vitro* screening results against parasitic protozoa.

Parasite / Samples/ Standards	*P. falciparum* K1 ~~(K1)~~	*T. b. rhodesiense*	*T. cruzi*	*L. donovani*
	(IC_{50}) µg/ml			
Chloroquine	0.0665			
Melarsoprol		0.00195		
Benznidazole			0.5175	
Miltefosine				0.1185
Melicopicine (1)	12.45	15.56	>30	>30
Normelicopicine (2)	3.35	5.24	30	1.08
Arborinine (3)	1.61	23.52	>30	5.20
Skimmianine (4)	5.60	15.78	14.50	9.40
β–Sitosterol (5)	8.20	>90	>30	3.80
α-Amyrin (6)	0.96	11.21	>30	7.90
Methanol crude extract	354.00	>90	30	>30

chloroquine diphosphate against chloroquine-resistance strain K1, stage IEF are shown in Table 3. The methanolic crude extracts had low activity of 354.00 µg/ml against *P. falciparum*. The compound melicopicine (1) also displayed low activity with IC_{50} of 12.45 µg/ml. Arborinine (3), normelicopicine (2), skimmianine (4) and β–sitosterol (5) showed mild activity with IC_{50} of 1.61, 3.35, 5.60 and 8.20 µg/ml, respectively. The standard, chloroquine, had an IC_{50} of 0.0665 µg/ml. α-Amyrin (6) was considered to have good activity with IC_{50} values of 0.96 µg/ml. Implying that α-amyrin had high ability to inhibit the growth of *P. falciparum* by preventing the uptake of [^{3}H]-hypoxanthine.

The potency of α-amyrin (6) to inhibit growth of *P. falciparum* is noteworthy. Although the compound activity was lower than that of chloroquine, an active anti-plasmodial drug used in malarial chemotherapy. Two compounds, 3 and 6 represent potential candidate for further *in vivo* analysis for their possible application or even template compounds for the synthesis of a suitable anti-malarial agent. Although, β-sitosterol (5) and α-amyrin (6) share a common biosynthetic pathway, α-Amyrin was about nine times as potent as β-sitosterol. Previously β-sitosterol had been reported to exhibit both antifungual and antibacterial activities against *Fusarium spp*. and *Salmonella typhii*, respectively (Kiprono et al., 2000). Similarly, the anti-plasmodial activity of the three-acridone alkaloids, arborinine (3), normelicopicine (2) and melicopicine (1) follows an interesting trend. Although, melicopicine has been reported to have antibacterial and antifungal activities against *Bacillus subtilis* and *Cladosporium curcumerinum*, respectively (Lwande et al., 1983).

Arborinine (3) was about twice as potent as normelicopicine (2) and eight-fold as melicopicine (1). Nevertheless, the activities of these compounds were relatively low compared to chloroquine resistance strain. Both compounds (2) and (3) possess 1-hydroxyl group in their structures which is lacking in compound (1), the hydroxyl group could be responsible for their anti-plasmodial activity, this is in agreement with earlier observation that methylation of the hydroxyl group resulted in marked loss of anti-plasmodial activity (Muriithi et al., 2002). The activities observed in the present study are higher then those reported by Muriithi et al. (2002) and this is due to different strains used in the bioassay.

Normelicopicine (2) showed moderate activity against *T. b. rhodesiense* (strain STIB 900, stage

trypomastigotes) with an IC_{50} value of 5.24 μg/ml. However, α-amyrin (6), skimmianine (4), arborinine (3) and melicopicine (1) showed lower activity with IC_{50} values of 11.21, 15.78, 23.52, and 15.56 μg/ml, respectively. The methanolic crude extract and β-sitosterol (5) were considered inactive with an IC_{50} value greater than 90 μg/ml being recorded. These results when compared to the results of melarsoprol (0.00195 μg/ml), it is observed that the compounds were of low activity.

Normelicopicine (2) and the methanolic crude extract exhibited moderate activity against *T. cruzi* (strain Tulahuen C4, stage trypomastigotes) with an IC_{50} of 30.0 and while skimmianine (4) showed an activity of 14.50 μg/ml. The other isolated compounds exhibited low or no activity with an IC_{50} value greater than 30 μg/ml being observed (Table 3). Comparing with the standard, benznidazole the isolated compounds displayed lower to no activity. Although arborinine (3) and skimmianine (4) had been reported to display moderate trypanocidal activities against (Y strain, stage trypomastigotes) with IC_{50} 1.231 and 1.455 μg/ml (Ambrozin et al., 2005). These result shows selectivity against different strains of *T. cruzi*.

Anti-trypanosomal activity of the three-acridone alkaloids showed that normelicopicine (2) displayed mild activities to both *T. b. rhodesiense* and *T. cruzi w*hile arborinine (3) and melicopicine (1) were mildly active to the former and considered inactive to the latter, thus displaying some selectivity.

Normelicopicine (2), β-sitosterol (5), arborinine (3), α-amyrin (6) and skimmianine (4) showed moderate activity against *L. donovani* (strain MHOM-ET-67, stage amastigotes) with IC_{50} values of 1.08, 3.80, 5.20, 7.90 and 9.40 μg/ml, respectively. Skimmianine (4) had been reported to display *in vitro* antileishmanial activity (4.25 μg/ml) against *L. amazonensis* (Allan et al., 1993). The methanolic crude extract and the alkaloid melicopicine (1) exhibited no activity with an IC_{50} value greater than 30 μg/ml being observed. Comparing these results with the standard, miltefosine with an IC_{50} value of 0.1185 μg/ml suggests that the tested compounds could be potential candidates for further analysis and may provide lead compounds for the synthesis of anti-leishmania agents.

The *in vitro* cytotoxicity assay results are summarized in Table 4, and expressed as minimum inhibitory concentration (MIC) in μg/ml. The cytotoxicity of the crude extracts and isolated compound was evaluated on myoblasts (L-6) cells. Arborinine (3) and skimmianine (4) exhibited moderate cytotoxicity towards L-6 cells with minimum inhibitory concentration (MIC) of 12.2 and 38.6 μg/ml, respectively. The methanolic crude extract and the other compounds had MICs more than 90 μg/ml which for the macrophage is an indication of low cytotoxicity.

Comparing with the standard, podophyllotoxin with MIC of 0.006 μg/ml the isolated compounds displayed low to no cytotoxicity towards L-6 cells. The cytotoxicity results could be used to predict a starting dose for an *in vivo* lethality assay and could also justify the usage of the plant in traditional medicine due its low toxicity.

Table 44. *In vitro* assay of MeOH crude extract and the isolated compounds against L-6 cells.

Samples/standard	Cytotoxicity L-6 cells (IC_{50}) μg/ml
Podophyllotoxin	0.006
Melicopicine (1)	>90
Normelicopicine (2)	>90
Arborinine (3)	12.2
Skimmianine (4)	38.6
β–Sitosterol (5)	>90
α-Amyrin (6)	>90
Methanol crude extract	>90

Conclusion

This study has shown that *T. trichocarpa* leaves contain acridone, furoquinoline alkaloids, and triterpenoids, in addition to other compounds. The compounds with low IC_{50} values against parasitic protozoa and high MIC values on myoblasts (L-6) cells have both demonstrated to be potent anti-protozoal compounds, suggesting that there may be an objective basis for traditional use of the *T. trichocarpa* against parasitic diseases. These compounds present potential candidate for further *in vivo* anti-protozoal and toxicological evaluation, backed by these *in vitro* results. Some of these compounds may find use as templates for the synthesis of anti-protozoal drugs.

ACKNOWLEDGEMENTS

The authors are grateful to AICAD for financially sponsoring this research (in part) under the contract number AICAD/RD-06/FPP/03-017 AICAD research fund and to Mr. H. M. Malebo for running the NMR spectra and for carrying out the anti-protozoal assay at the Swiss Tropical Institute (STI), Basel.

REFERENCES

Allan F, Alcira AB, Victoria M, Reynald H, Andre C, Jean B (1993). 2-Substituted quinoline alkaloids as potential antileishmanial drugs. Antimicrob. Agents Chemother. 37: 859-963.

Ambrozin ARP, Mafezoli J, Vieira PC, Fernandes JB, Silva MFGF, Ellena JA, Albuquerque S (2005). New pyrone and quinoline alkaloid from *Almeidea rubra* and their trypanocidal actvities. J. Br. Chem. Soc. 16: 434-439.

Bergenthal D, Mester I, Rózsa Z, Reisch J (1979). ^{13}C-NMR-spektren einiger acridon-alkaloide. Phytochem. 18: 161-163.

Bryceson ADM (1996). Leishmaniasis. In: Cook GC (ed) Manson's

Tropical Diseases, 20th edn, London: W.B. Saunders and Co. pp. 1213-1245.
Cunningham I (1977). New culture medium for maintenance of tsetse tissues and growth of trypanosomatids. J. Protozool. 24: 325-329.
Howard RJ (1987). Vaccination against malaria: Recent advances and the problems of antigenic diversity and other parasite evasion mechanism. Int. J. Parasitol. 17: 17-29.
Houghton PJ (2001). Old yet new-pharmaceuticals from pants. J. Chem. Edu. 78: 175-184.
Kain KC (1995). Chemotherapy and prevention of drug-resistant malaria. Wilderness Environ. Med. 6: 307-324.
Kiprono CP, Kaberia F, Karanja JN, Keriko JM (2000). The *in vitro* antifungal and antibacterial activities of β-sitosterol from *Senecio lyratus* (Asteraceae). Z. Naturforsh. 55: 485-488.
Lwande W, Gebreyesus T, Chapya A, Macfoy C, Hassanali A, Okech M (1983). 9-Acridone insect antifeedant alkaloids from *Teclea trichocarpa* bark. Insect Sci. Appl. 4: 393-395.
Mahato SB, Kundu AP (1994). ^{13}C NMR spectra of pentacyclic triterpenoids–a complilation and some salient features. Phytochem. 37: 1517-1575.
Mascolo N, Pinto A, Yenesew A, Dagne E (1988). Antipyretic and analgesic studies of the ethanolic extract of *Teclea nobilis* Delile. Phytother. Res. 2: 154-156.
Matile H, Pink JRL (1990). *Plasmodium falciparum* malaria parasite cultures and their use in immunology. In: Lefkovits, I., Pernis, B. (eds.). Immunological Methods. San Diego: Academic press pp. 221-234.
Molyneux DH, Pentreath V, Doua F (1996). African trypanosomiasis in man. In: Cook GC (ed) Manson's Tropical Diseases, 20th edn, London: W.B. Saunders and Co. pp. 1171-1196.
Muriithi MW, Abraham WR, Addae-Kyereme J, Scowen I, Croft SL, Gitu PM, Kendrick H, Njagi ENM, Wright CW (2002). Isolation and *in vitro* antiplasmodial activities of alkaloids from *Teclea trichocarpa*: *in vivo* antimalarial activity and x-ray crystal structure of normelicopicine. J. Nat. Prod. 65: 956-959.
Olliaro P, Cattani J, Wirth D (1996). Malaria: the submerged disease. JAMA 275: 230-234.
Rasoanaivo P, Federici E, Palazzino G, Galeffi C (1999). Acridones of *Vepris sclerophylla*: their ^{13}C NMR data. Fitoterapia 70: 625-627.
Räz B, Iten M, Grether-Buhler Y, Kaminsky R, Brun R (1997). The Alamar blue assay to determine drug sensitivity of African trypanosomes (*Trypanosoma brucei rhodesiense* and *T. b. gambiense*) Acta Trop. 68: 139-147.
Ridley RG, Hofheinz W, Matile H, Jaquet C, Dorn A, Masciadri R, Jolidon S, Richter WF, Guenzi A, Girometta MA, Urwyler H, Huber W, Thaithong S, Peters W (1996). 4-Aminoquinoline analogs of chloroquine with shortened side chains retain activity against chloroquine-resistant *Plasmodium falciparum*. Antimicrob. Agents Chemother. 40: 1846-1854.
Watt JM, Breyer-Brandwijk MG (1962). The medicinal and poisonous plants of Southern and Eastern Africa. 2nd ed, Edinburgh: E. & S. Livingstone Ltd p. 923.
WHO (1984). Chemical Methods for the Control of Arthropod Vectors and Pests of Public Health Importance. Geneva: World Health Organization p. 108.
WHO (2002). The World Health Report 2002: Reducing Risks, Prompting Healthy Life. Geneva: World Health Organization p. 248.
Wright CW, Phillipson JD (1990). Natural products and the development of selective antiprotozoal drugs. Phytother. Res. 4: 127-139