Investigating South African plants as a source of new antimalarial drugs

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Abstract

Based on the historical success of natural products as antimalarial drugs and the urgent need for new antimalarials, a number of South African medicinal plants have been evaluated for their antimalarial properties. This paper reviews the major studies conducted and their findings. Overall three ethnobotanical screening programmes have been conducted on South African plants and there have been three studies adopting a more direct approach where plants within a particular genus were screened for antiplasmodial activity. The paper also summarizes antimalarial plants, which were studied individually, as well as the bioactive molecules identified from selected active plant extracts.

Keywords: malaria, ethnopharmacology, antiplasmodial screening, bioassay-guided fractionation, sesquiterpene lactones; cytotoxicity

Contents

1. Malaria and the situation in South Africa

Malaria, caused by parasites of the genus *Plasmodium*, is one of the leading public health problems in Sub-Saharan Africa responsible for over a million deaths annually

(WHO, 2005). This is due to the majority of infections in Africa being caused by *Plasmodium falciparum*, the most dangerous of the human malaria parasites as well as the most effective and difficult to control malaria vector, the mosquito *Anopheles gambiae*, which is the most widespread in Africa. Climatic conditions over a large part of Africa favour malaria transmission and global warming together with changes in land use are extending the areas of transmission.

In South Africa transmission is currently restricted to the low-altitude regions of Kwazulu-Natal, Mpumulanga and Limpopo; three provinces in the north-eastern part of the country (along the border with Mozambique and Swaziland). Malaria transmission in South Africa is seasonal with the greatest number of cases occurring between October and May with a significant inter-annual variation in the number of malaria cases. In the year 2005 the annual number of reported malaria cases was approximately 7 755 while in 2006 it was 12 098 (Department of Health, 2007). This variation is mainly attributed to favourable climatic conditions, population migration and the emergence of drug resistant parasites. The South African government has increased efforts to control malaria particularly due to its devastating impact on the economy and the threat of increased prevalence and distribution, primarily due to the emergence and spread of drug resistant parasites. Research efforts are subsequently being directed towards the discovery and development of affordable drugs with different structural features.

2. The role of ethnopharmacology in the fight against malaria

The main goal of ethnopharmacology is to discover novel plant-derived compounds, based on the indigenous use of medicinal plants, which can be developed into new

pharmaceuticals. Historically, plants have proven to be a major source of drugs (Newman et al., 2003), with two of the most widely used antimalarials originating from plants – the quinoline-based antimalarials are modelled on quinine, derived from the bark of the Peruvian *Cinchona L*. tree; and the endoperoxide-based antimalarials originated from artemisinin, first isolated from the Chinese herbal medicine *Artemisia annua* L (Camacho et al., 2000). In light of this historic success and the fact that most indigenous people living in malaria endemic areas use traditional medicines to fight this disease, there is every possibility that ethnopharmacological approaches could lead to new antimalarial agents (Phillipson and Wright, 1991).

The development of continuous culturing of *P. falciparum* (Trager and Jensen, 1976) and subsequent *in vitro* assays (Desjardins et al., 1979; Geary et al., 1983; Makler et al., 1995) made it possible to screen plant extracts for antiplasmodial activity and use bioassay-guided fractionation to isolate active principles (Schwikkard and van Heerden, 2002). A number of studies have applied an ethnobotanical approach to investigate plants from malaria endemic areas as a source of novel antimalarial drugs (Gessler et al., 1994; Kraft et al., 2003, Katuura et al., 2007; Soh et al., 2007). South Africa's rich biodiversity and long history of traditional medicinal plant use has also prompted several studies to evaluate local indigenous plants for antimalarial properties (Prozesky et al., 2001; Nundkumar and Ojewole, 2002; Clarkson et al., 2004). This review aims to consolidate the major findings of these screening programmes as well subsequent bioassay-guided fractionation studies to assess the potential of South African plants for the discovery of new antimalarial drugs.

3. Methodology

In order to source information on studies, other than that of the authors, a Pubmed and Google Scholar search was conducted using key words (malaria, South Africa, antimalarial / antiplasmodial, medicinal plants, traditional medicine, plant extract). Articles and relevant references were selected based on ethnobotany, *in vitro* or *in vivo* antimalarial activity and toxicity of South African plants, focusing on endemic species and studies conducted by local researchers.

All publications cited use classical methodologies such as the continuous culture of *Plasmodium falciparum* (Trager and Jensen, 1976), *in vitro* antiplasmodial tests based on parasite lactate dehydrogenase (pLDH) activity (Makler et al., 1995), flow cytometric activity (Schulze et al., 1997) or the titrated hypoxanthine incorporation method (Desjardins et al., 1972); and *in vitro* cytotoxicity measured on known cell-lines (*viz*. vervet monkey kidney cells and Chinese Hamster Ovarian, CHO, cells) using standard protocols such as the MTT assay (Mosmann, 2003).

4. Results and Discussion

Overall three ethnobotanical screening programmes were conducted on South African plants (Prozesky et al., 2001; Nundkumar and Ojewole, 2002; Clarkson et al., 2004). There were three further studies where a more direct approach was adopted and plants within a particular genus were screened for antimalarial activity (Van Zyl and Viljoen, 2002; Kamatou et al., 2005; Kamatou et al., 2008). In addition several specific plants were studied individually (Campbell et al., 2000; Clarkson et al., 20003; Kamdem Waffo et al., 2007). Table 1 summarizes all plant taxa that have been bioassayed for antiplasmodial properties in South Africa. Several molecules were also

identified from selected active plant extracts. Table 2 summarises the biological assaying data of the isolated compounds.

Prozesky et al. (2001) screened 14 ethnobotanically described antimalarial plants, collected from Venda and Northern Kwazulu-Natal, against a chloroquine-resistant (PfUP1) strain of *Plasmodium falciparum* using the flow cytometric method (Schulze et al.). Nine of these (64%) were found to have an IC_{50} below 5 μg/ml. The two most active extracts were the dichloromethane stem bark extracts of *Ozorea engleri* R.A. Fernandes (Anacardiaceae) (IC_{50} 1.70 μg/ml) and *Balantines maughamii* Sprague (Balanitaceae) (IC_{50} 1.94 μg/ml). Little is known about the medicinal properties of these plants but both are related to Tanzanian species with antimalarial activity i.e. *O. insignis* (Gessler et al., 1994) and *B. aegyptiaca* (Weenen et al., 1990). The authors concluded that the extracts had poor selectivity based on the ratio of *in vitro* antiplasmodial activity to cytotoxicity (monkey kidney cell test) but warranted further investigation based on the findings of Kirby et al., (1993) that *in vitro* cytotoxicity is not always a clear indication of toxicity *in vivo*. However there were no follow-up studies reported on the active plant extracts identified in this study.

Based on an ethnobotanical literature survey and interviews with traditional healers from KwaZulu-Natal, Nundkumar and Ojewole (2002) collected and screened seven plants that are frequently used as antimalarial remedies in Zulu folk medicine. The extracts were bioassayed against a chloroquine-sensitive D10 strain of *P. falciparum* using the pLDH assay. None of the extracts had an IC_{50} below 10 μg/ml, with chloroquine, itself, showing an unusually high IC_{50} of 6 μg/ml in this study. The two most active extracts were the *Psidium guajava* L. (Myrtaceae) stem-bark and

Vangueria infausta Burch (Rubiaceae) leaf extracts, both of which showed IC_{50} values of 10 - 20 μg/ml.

The leaves of *P. guajava* are used as an ingredient in the preparation of fever "teas" and they are also used as part of the pot herb used in steam treatment for malaria (Iwu, 1993). Olajide et al. (1998) speculated that the plants use in malaria and fevers could be attributable to it anti-inflammatory and antipyretic activities, which they established using a methanol extract of the leaves of *P. guajava*. However, Nundkumar and Ojewole (2002) did detect moderate antiplasmodial activity in the aqueous stem-bark extract of the plant.

An infusion of the roots and leaves of *V. infausta* has been used to treat malaria (Watt and Breyer-Brandwyk, 1962). Following up on the *in vitro* antiplasmodial efficacy of the aqueous leaf extract reported by Nundkumar et al. in 2002, Abosi et al. (2006) recently demonstrated the *in vitro* activity of the root bark extract of *V. infausta* using the 3 H-hypoxanthine uptake method (Desjardins et al., 1979). The root bark extract was also shown to have antimalarial activity against *Plasmodium berghei* in mice, exhibiting 73.5% suppression in early infection and a repository effect of 88.7% . The plant showed the presence of flavanoids, coumarins, tannins, terpenoids, anthraquinones and saponins (Abosi et al., 2006). .

Van Zyl and Viljoen (2002) adopted a more selective approach and screened 34 *Aloe* species and their main constituents for antiplasmodial activity using the titrated hypoxanthine incorporation assay (Desjardins et al., 1972). Aloes are not commonly known to possess antimalarial activity but this study was prompted by reports on the

use of *A. secundiflora* and *A. lateritia* for treating malaria related symptoms (Neuwinger, 1996) as well as the *in vivo* non-specific immunostimulant clearing of a *P. Berghei* malaria infection within two days when *A. vaombe* extract was used prophylactically before the mice were inoculated (Brossat et al., 1981). The 34 species tested showed variable antimalarial activity with the leaf extracts of *A. viriflora*, *A. wickensii*, *A. spicosa* and *A. suprafoliata* exhibiting the most promising activity. The methanol extracts were found to have relatively high IC_{50}'s (Table 1), with *A. viridiflora* being the most active (IC_{50} ~ 32 μg/ml, and were shown to have limited toxicity to kidney epithelium cells at similar concentrations.(Van Zyl and Viljoen, 2002). C-glucoside homonataloin (**1**), the common active species, was found to have an IC_{50} of 13.46 μg/ml; while aloin (**2**) was significantly less active with an IC_{50} of 107.20 μg/ml (Table 2).

In light of the results from these preceding studies and the urgent need for new antimalarials, the South African Department of Arts, Culture, Science and Technology (now the Department of Science and Technology) awarded an innovation fund to a national multi-disciplinary consortium to conduct a more extensive evaluation of the antimalarial activity of local medicinal plants. Weighted criteria (primarily ethnobotanical and chemotaxanomic) were used to rank and select from the 623 taxa, associated with malaria and/or fever, occurring indigenously or naturalised within the FSA region. From the ranked list over 134 species, representing 54 families, were collected throughout South Africa and extracts thereof were tested for *in vitro* activity against the D10 *P. falciparum* strain using the pLDH assay (Table 4). Sixty-six species (49%) were reported to show promising antiplasmodial activity

(IC50 ≤ 10 μg/ml), of which 17% (23 species) were considered highly active (IC50 ≤ 5 μg/ml) (Clarkson et al., 2004).

Several plant species and genera, i.e. *Euclea* (Ebenaceae); *Kirkia* (Kirkiaceae); *Pittosporum* (Pittosporaceae); *Ranununculus* (Ranunculaceae) and *Setaria* (Poaceae), were shown for the first time to possess *in vitro* antiplasmodial activity. Despite being the subject of substantial phytochemical research, species such as *Catha edulis* (Vahl) Forssk. Ex. Endl. (Celastraceae) and *Ocimum americanum* L. var. *americanum* (Lamiaceae), were not previously reported to show antiplasmodial activity. In addition to identifying further species within genera viz. *Vernonia* (Asteraceae) that display activity (*e.*g. *V. colorata*; *V. myriantha* and *V. oligocephala*) our findings were substantiated by earlier reported antiplasmodial activity elsewhere in the genera (Alves et al., 1997; Oketch-Rabah et al.; Abosi and Raseroka, 2003; Kraft et al., 2003).

Similarly, our results (Clarkson et al., 2004) for species like *Trichilia emetica* Vahl (Meliaceae) and *Artemisia afra* jacq. ex Willd. (Asteraceae) agreed with previous reports on their antiplasmodial activity (El Tahir et al., 1999; Prozesky et al., 2001; Kraft et al., 2003). Results for *Rouvolfia caffra* Sond. (Apocynaceae), *Barringtonia racemosa* (L.) Roxb. (Lecythidaceae) and *Vanguera infausta* Burch (Rubiaceae) supported the relatively low antiplasmodial activity (IC_{50} > 10 μg/ml) reported by Nundkumar and Ojewole (2002) for these species. On the other hand, the activity of *Aloe marlothii* A. Berger (Asphodelaceae) was found to be considerably higher (IC_{50} 3.5 μg/ml) than that reported previously (IC_{50} > 50 μg/ml) by Van Zyl and Viljoen (2002), while the results for *Ziziphus mucronata* Willd. (Rhamnaceae) was found to

be much lower than that reported by Prozesky et al. (2001). However, the difference in plant part tested and extraction procedures as well as geographical and seasonal variation can account for the differences in antiplasmodial activity observed between studies.

The active taxa (IC_{50} < 5 μg/ml) identified by our study (Clarkson et al.; 2004) were subsequently screened against a chloroquine-resistant K1 strain and prioritised for bioassay-guided fractionation based on activity and results of literature studies. Plants such as *Catha edulis* (Vahl) Forssk. Ex. Endl. (Celastraceae), which is known to contain amphetamine-like alkaloids, and *Pittosporum viridiflorum* Sims (Pittosporaceae) which is rich in false-positive saponins, were excluded. In most cases plants prioritised for further study needed to be recollected, and as far as possible this was carried out from the original collection site. However, in several instances the original activity could not be reproduced. Several extracts also lost activity during the fractionation process (viz. *Artemisia afra* and *Trichilia emetica*), which may be attributable to the instability of the actives or the loss of the synergism upon fractionation.

The bioassay-guided fractionation of taxa led to known actives in cases such as *Plumbago zeylanica* L. (Plumbaginaceae), where the active ingredient was identified as plumbagin (**3**), the antiplasmodial activity of which is already reported (Likhitwitayawuid et al., 1998). A few of the plants identified by the consortium-based screening programme (Clarkson et al., 2004) are still under investigation and will be reported at a later stage.

Table 1 includes two species, *Vernonia staehelinoides* Harv (Asteraceae) and *Oncospihon piluliferum*, (L.f.) Kallersjo (Asteraceae) which also stemmed from the Clarkson et al. (2004) screening programme; these were not included in the original publication but formed the basis of two subsequent reports (Pillay et al, 2007a ; b). Bioassay-guided fractionation of the active extracts of these plants led to the isolation of sesquiterpene lactones derivatives with good antiplasmodial activity but limited selectivity (bioactivity *vs*. cytotoxicity).

Vernonia staehelinoides Harv. (Asteraceae) is reported to be used medicinally but no details have been specified (Watt and Breyer-Brandwyk, 1962). The dichloromethane extract of the leaves of *V. staehelinoides* showed equipotent *in vitro* activity (IC_{50} ~3 µg/ml) against the chloroquine-sensitive (D10) and the chloroquine-resistant (K1) strains of *Plasmodium falciparum* (Pillay et al., 2007a). The active components were identified as two structurally-related hirsutinolides (**4)** and (**5)**, previously isolated from other South African *Vernonia* species (Bohlmann et al., 1983; Tully et al., 1987), but were reported for the first time to display *in vitro* antiplasmodial activity (IC_{50} ~0.2 µg/ml against D10). The compounds were found to be cytotoxic to mammalian Chinese Hamster Ovarian (CHO) cells at similar concentrations but proved to be attractive scaffolds for structure-activity relationship studies. The 2(*5H*)-furanone substructure was identified as a key pharmacophore in the observed antiplasmodial activity, the synthesis and antiplasmodial activity of a range of analogues based on this substructure is anticipated to be reported in due course.

Oncosiphon piluliferum (L.f.) Kallersjo (*Asteraceae*) is used traditionally to treat a variety of ailments, mainly fevers. The dichloromethane extract of the aerial parts of

the plant showed activity *in vitro* against the chloroquine-sensitive (IC_{50} 2.6 µg/ml) and the chloroquine-resistant (IC_{50} 3.1 µg/ml) strains of *Plasmodium falciparum*. (Pillay et al., 2007b). Bioassay-guided fractionation identified sesquiterpene lactones (**6**, **7**, **8, 9** and **10**) with significant *in vitro* antiplasmodial activity (IC_{50} values ranging from 0.4 to 4.4 µg/ml. In addition, the cytotoxic effects of the active compounds against Chinese Hamster Ovarian (CHO) cells were evaluated and the compounds were found to be toxic to mammalian cells at similar concentrations. The sesquiterpene lactones were of the germacranolide and eudesmanolide type and have all been reported to occur in various other members of Asteraceae (Shafizadeh and Bhadane, 1973; Bohlmann et al., 1982; Jakupovic et al., 1988; Gören et al., 1992; *Yunusov* et al., 1979; Yunusov et al., 1976; Sanz and Marco, 1991; Izbosarov et al., 2000) but this (Pillay et al., 2007b) was the first report of any of them having antiplasmodial properties.

Salvia species (sage) is well known as a folk-medicine and is used to treat fevers and digestive disorders in South Africa. Three closely related South African species (*S. stenophylla*, *S. repens* and *S. runcinata*) were evaluated for their antimalarial properties (Kamatou et al., 2005) using the titrated hypoxanthine incorporation assay (Desjardins et al., 1979; Van Zyl and Viljoen, 2002). The methanol extracts were found to exhibit moderate antimalarial activity (IC_{50}'s ~ 17.0, 29.0 and 78.9 µg/ml; respectively) compared with the essential oils (IC_{50}'s ~ 4.4, 1.2 and 1.7 µg/ml; respectively). The essential oils were found to be cytotoxic at similar concentrations using the MTT assay (Mosmann, 1983; van Zyl and Viljoen, 2002). The bioactivity and cytotoxicity was attributed to the presence of sesquiterpenes such as nerolidol

(**11**), which is reported to have antimalarial (Lopes et al., 1999) and cytotoxic properties (van Zyl and Viljoen, 2003).

In a recent follow-up study (Kamatou et al., 2008) solvent extracts of seventeen *Salvia* species (Lamiaceae) used in traditional medicine in South Africa were evaluated for their ability to inhibit the *in vitro* growth/proliferation of *Plasmodium falciparum* (chloroquine-resistant FCR-3 strain) using the [^{3}H]-hypoxanthine method. The extracts displayed antimalarial activity with IC_{50} values ranging from 3.91 to 26.01 μg/ml. Of note, *S. repens*, was shown to have an IC_{50} of 8.25 ± 2.09 μg/ml which is comparable to that demonstrated by Clarkson et al. (2004) (IC_{50} = 10.8 μg/ml) using the pLDH assay. As *S. radula* exhibited the best activity ((IC_{50} = 3.91 ± 0.52 μg/ml) from the plants screened by Kamatou et al. (2008), it was subjected to bioassay-guided fractionation. Two compounds were subsequently isolated from the active fraction of *S. radula* and were identified as betulafolientriol oxide (**12**) and salvigenin (**13**). The two compounds displayed similar or lower activity (IC_{50} values of 4.95 and 24.60 μg/ml; respectively) compared to the crude extract.

Methanol extracts of the dried leaves and rhizome of *Albertisa delagoensis* N.E.Br.Forman (Menispermaceae), used as an antipyretic, were tested on a chloroquine-resistant Gambian FCR-3 strain of *P. falciparum* and exhibited IC_{50}'s of 4.1 μg/ml and 1.6 μg/ml; respectively (De Wet et al., 2007). The extracts were tested for cytotoxicity against the Graham cell line (transformed human kidney epithelium cells); the leaves showed limited toxicity and gave an IC_{50} of 166 μg/ml or a growth inhibition of 2.5% at 200 μg/ml, while the rhizomes had an IC_{50} of 166 μg/ml or a growth inhibition of 93.9% at 200 μg/ml. Bioassay-guided fractionation was not

conducted but the authors (De Wet et al., 2007) reported on the isolation of alkaloid constituents of the leaf and rhizome extracts *viz*. cocsoline (**14**) and concluded that the medicinal properties of *A. delagoensis* can probably be explained by the alkaloids present in this plant. The selectivity of the leaf extract shows potential for this species as an antimalarial.

4. Conclusions

Overall the results of the screening programmes supported a rational rather than random approach to the selection of antiplasmodial screening candidates and identified a number of promising taxa for further investigation as plant based antimalarial agents.

The identification of compounds with antiplasmodial properties from South African medicinal plants implicated in the treatment of malaria suggests that they may play a role in the medicinal properties of the plant, but their potential for the development of antimalarial drugs is limited due to inherent cytotoxicity and lack of selectivity. This is often the case with antimalarial compounds identified from plants (Schwikkard and van Heerden, 2002).

In considering a recent publication (Pink et al., 2005) outlining criteria for antiparasitic drug discovery, a compound can be considered a hit if it is:

- Active *in vitro* against whole protozoa with an IC_{50} of $\leq 1 \mu g/ml$
- Selective (at least tenfold more active against the parasite than against a mammalian cell line)

Based on these criteria, only compounds (**4**), (**9**) and (**10**) can be considered hits (Table 2). Although their activity and selectivity cannot be compared to that of chloroquine, these compounds could potentially be subjected to more detailed evaluation involving accurate IC_{50} determinations against different strains of the parasite, measurement of general toxicity (using a range of mammalian cell lines) and *in vivo* assessment in animal models. The compounds could also be used as scaffolds for structure-activity relationship studies.

References

Abosi, A.O., Raseroka, B.H., 2003. *In vivo* antimalarial activity of *Vernonia amygdalina*. British Medical Journal of Biomedical Science 60, 89-91.

Abosi, A.O., Mbukwa, E., Majinda,R.R.T., Raseroka, B.H., 2006. *Vangueria infausta* root bark: in vivo and in vitro antiplasmodial activity, British Journal of Biomedical Science 63, 129-133

Alves, T.M., Nagem, T.J., de Carvalho, L.H., Krettli, A.U., Zani, C.L., 1997. Antiplasmodial triterpenes from *Vernonia brasiliana*. Planta Medica 63, 554-555.

Bohlmann, F., Ates (Gören), N., Jakupovic, J., 1983. Hirsutinolides from South African *Vernonia* species. Phytochemistry 22, 1159-1162.

Bohlmann, F., Alder, A., Jakupovic, J., King, R.M., Robinson, H., 1982. A dimeric germacranolide and other sesquiterpene lactones from *Mikania* Species. Phytochemistry, 21, 1349-1355.

Brossat, J.Y., Leduaut, J.Y., Ralamboranto, L., Rakotovao, L.H., Solar, S., Gueguen, A., Coulanges, P., 1981. Immunostimulating properties of an extract from *Aloe vahombe* . Archives de L'Institut Pasteur de Madagascar 72, 43-46.

Camacho, M.d.R., Croft, S.L., Phillipson, J.D., 2000. Natural products as sources of antiprotozoal drugs. Current Opinion in Anti-infective Investigational Drugs 2, 47-62.

Clarkson, C., Campbell, W.E., Smith, P., 2003. *In vitro* antiplasmodial activity of abietane and totarane diterpenes isolated from *Harpagophytum procumbens* (Devil's Claw). Planta Medica 8, 720-724.

Clarkson, C., Maharaj V.J., Crouch N.R., Grace O.M., Pillay P., Matsabisa M.G., Bhagwandhin N., Smith P.J., Folb P.I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. Journal of Ethnopharmacology 92, 177-191.

Department of Health, 2007. Malaria cases in South Africa - 1999 – 2007 (Annual) http://www.doh.gov.za/facts/index.html

Desjardins, R.E., Canfield, C.J., Haynes, J.D., Chulay, J.D., 1979. Quantitative assessment of antimalarial activity in vitro by a semi-automated microdilution technique. Antimicrobial Agents and Chemotherapy 16, 710-718.

De Wet, H., Van Heerden, F., Van Wyk, B, Van Zyl, R., 2007. Antiplasmodial activity and cytotoxicity of *Albertisia delagoensis*. Fitoterapia 78, 420-422.

El Tahir, A., Satti, G.M., Khalid, S.A., 1999. Antiplasmodial activity of selected Sudanese plants with emphasis on *Maytenus senegalensis* (Lam.) Exell. Journal of Ethnopharmacology 64, 227-233.

Fennel, C.W., Lindsey, K.L., McGraw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205-217.

Geary, T.G., Divo, A.A., Jensen, J.B, 1983. An in vitro assay system for the identification of potential antimalarial drugs. Journal of Parasitology 69 (3), 577-583.

Gessler, M.C., Nkunya, M.H.H., Mwasumbi, L.B., Heinrich, M., Tanner, M., 1994. Screening Tanzanian medicinal plants for antimalarial activity. Acta Tropica 56, 65-77.

Gören, N., Bozak-Johansson, C., Jakupovic, J., Lin, L., Shieh, H., Cordell, G.A., Celik, N., 1992. Sesquiterpene lactones with antibacterial activity from *Tanacetum Densum* subsp. *Sivascium*. Phytochemistry 31, 101-104.

Iwu, M.M., 1993. Handbook of African Medicinal Plants, C.R.C Press, Florida, p. 26–267.

Izbosarov, M.B., Zairova Kh. T., Abduazimov, B.Kh., Malikov, V.M., 2000. Aminoderivatives of sesquiterpene lactones from tanachin and tavulin and their biological activity. Chemistry of Natural Compounds 36, 288-291.

Jakupovic, J., Aad, M.A., Eid, F., Bohlmann, F., El-Dahmy, S., Sarg,T., 1988. Further glaucolides and other sesquiterpene lactones from *Brocchia Cinerea*. Phytochemistry 27, 2219-2223.

Kaatura, E., Waako, P., Tabuti, J.R.S., Bukenya-Ziraba, R., Ogwal-Okeng, J., 2007. Antiplasmodial activity of extracts of selected medicinal plants used by local communities in western Uganda for the treatment of malaria. African Journal of Ecology, 45 (Suppl. 3), 94-98.

Kamatou, G.P.P., Viljoen, A.M., ono-Bwalya, A.B., van Zyl, R.L., van Vuuren, S.F., Lourens, A.C.U, Baser, K.H.C., Demirci, B., Lindsey, K.L., van Staden, J, Steenkamp., P., 2005. The in vitro pharmacological activities and a chemical investigation of three South African *Salvia* species. Journal of Ethnopharmacology 102, 382-390.

Kamatou, G.P.P., Van Zyl, R.L., Davids, H., Van Heerden, F.F., Lourens, A.C.U., Viljoen, A.M., 2008. Antimalarial and anticancer activities of selected South African Salvia species and isolated compounds from *S. radula*. South African Journal of Botany 74, 238-243.

Kraft, C., Jennet-Siems, K., Siems, K., Jakupovic, J., Mavi, S., Bienzle, U., Eich, E., 2003. *In vitro* antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytotherapy Research 17, 123-128.

Likhitwitayawuid K., Kaewamatawong R., Ruangrungsi, N., Krungkrai, J., 1998. Antimalarial napthoquinones from *Nepenthes thorelii*. Planta Med 64, 237-241.

Lopes, N.P., Kato, M.J., Andrade, E.H.A., Maia, J.G.S., Yoshida, M., Planchart, A.R., Katzin, A.M., 1999. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiapi Amazon Indians. Journal of Ethnopharmacology 67, 313-319.

Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbons, B.L., Hinrichs, R.C., 1995. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. American Journal of Tropical Medicine and Hygiene 48, 739-741.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65, 55-63.

Neuwinger, H.D., 1996. African Ethnobotany. Poisons and Drugs. Chapman & Hall, London, pp 3-9.

Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural Products as sources of new drugs over the period 1981-2002. Journal of Natural Products 66, 1022-1037.

Nundkumar, N., Ojewale, J.A.O., 2002. Studies on the Antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu folk medicine. Methods and Findings in Experimental and Clinical Pharmacology 24 (7), 397-401.

Olajide, O.A., Awe, S.O., Makinde, J.M., 1999. Pharmacological studies of *Psidium guajava*. Fitoterapia 70, 25-31.

Oketch-Rabah, .A., Christensen, S.B., Frydenvang, K., Dossaji, S.F., Theander, T.G., Cornett, C., Watkins, W.M., Kharazmi., A., Lemmich., E., 1998. Antiprotozoal properties of 16,17-dihydrobrachycalyxolide from *Vernonia brachycalyx*. Planta Medica 64, 559-562.

Phillipson J.D., Wright, C.W., 1991. Can ethnopharmacology contribute to the development of antimalarial agents? Journal of Ethnopharmacology 32, 155-165.

Pillay, P., Vleggaar, R., Maharaj, V.J., Smith, P.J., Lategan, C.A., Chouteau, F., Chibale, K., 2007a. Antiplasmodial hirsutinolides from *Vernonia staehelinoides* and their utilisation towards a simplified pharmacophore. Phytochemistry 68, 1200-1205.

Pillay, P., Vlegaar, R., Maharaj, V.J., Smith, P.J., Lategan, C.A., 2007b. Isolation and identification of antiplasmodial sesquiterpene lactones from *Oncosiphon piluliferum*. Journal of Ethnopharmacology 112, 71-76.

Prozesky, E.A., Meyer, J.M.M., Louw, A.I., 2001. In vitro antiplasmodial activity and cytotoxicity of ethnobotanically selected South African plants. Journal of Ethnopharmacology 76, 239-245.

Sanz, J.F., Marco, J.A., 1991. NMR studies of tatridin A and some related sesquiterpene lactones from *Tanacetum vulgare*. Journal of Natural Products 54, 591-596.

Schulze, D.L.C., Makgatho, E.M., Coetzer, T.L., Louw, A.I., Van Rensburg, C.E.J., Visser, L., 1997. Development and application of a modified flow cytometric procedure for rapid in vitro quantification of malaria parasitaemia. South African Journal of Science 93, 156-158.

Shafizadeh, F., Bhadane, N.R., 1973. Sesquiterpene lactones of *Artemisia Arbuscula* and *A. Tridentata*. Phytochemistry 12, 857-862.

Schwikkard, S., van Heerden, F.R., 2002. Antimalarial activity of plant metabolites. Natural Product Reports 19, 675-692.

Soh, P.N., Benoit-Vical, F., 2007. Are West African plants a source of future antimalarial drugs? Journal of Ethnopharmacology 114, 130-140.

Trager, W., Jensen, J.B., 1976. Human malaria parasites in continuous culture. Science 193, 673-675.

Tully, L.E., Carson, M.S., McMurray, T.B.H., 1987. A novel sesquiterpene lactone from *Vernonia erinacea*. Tetrahedron Letters 28, 5925-5928.

Van Zyl, R.L., Viljoen, A.M., 2002. In vitro activity of *Aloe* extracts against Plasmodium falciparum. South African Journal of Botany 68, 106-110.

Van Zyl, R.L., Viljoen, A.M., 2003. Antimalarial activity and essential oil composition of South African medicinal aromatic plants. South African Journal of Botany 69, 265.

Watt, J.M. and Breyer-Brandwyk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingston, London, pp. 254.

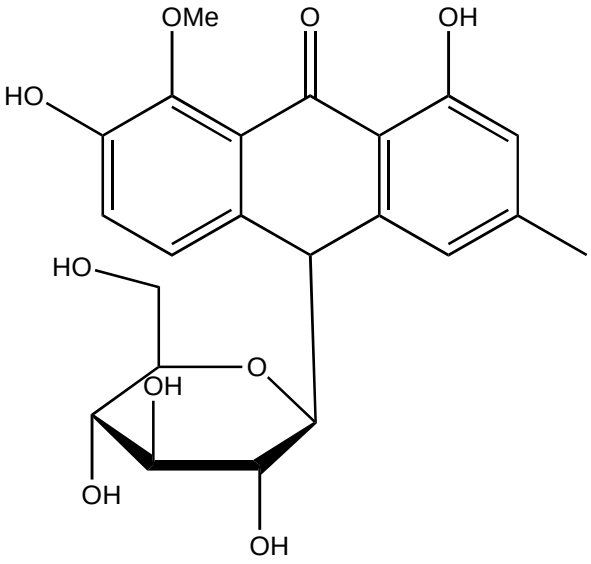
Weenen, H., Nkunya, M.H.H., Bray, D.H., Mwasumbi, L.B., Kinabo, L.S., Kilimali, V.A.E.B., 1990. Antimalarial activity of Tanzanian medicinal plants, Planta Medica 56, 369.

World Health Organisation, 2005. World Malaria Report http://www.rbm.who.int/wmr2005/pdf/adv_e.pdf

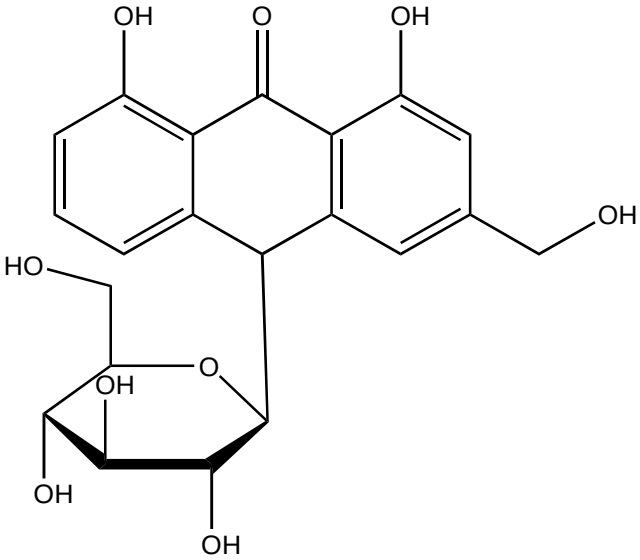
Yunusov, A.I., Abdullaev, N.D., Kasymov, S.Z., Sidyakin, G.P., 1976. Tanachin, a new sesquiterpene lactone from *Tanacetum Pseudochillea*. Chemistry of Natural Compounds 2, 236-237.

Yunusov, A.I., Abdullaev, N.D., Kasymov, S.Z., Sidyakin, G.P., Tagudaev, M.R., 1976. Structure of tanachin. Chemistry of Natural Compounds 4, 462-467.

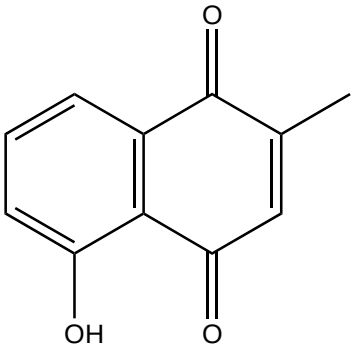
Yunusov, A.I., Sidyakin, G.P., Nigmatullaev, A.M., 1979. Sesquiterpene Lactones of *Tanacetum Vulgare*. Chemistry of Natural Compounds 1, 101-102.



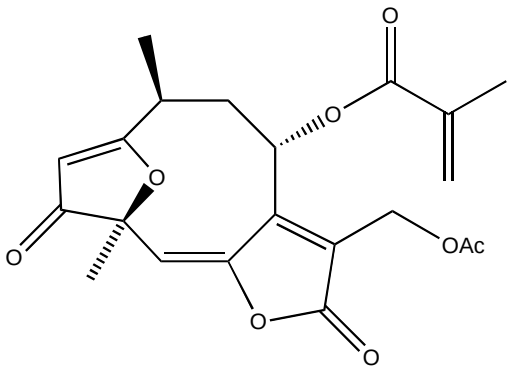
Homonataloin **(1)**



Aloin **(2)**



Plumbagin **(3)**



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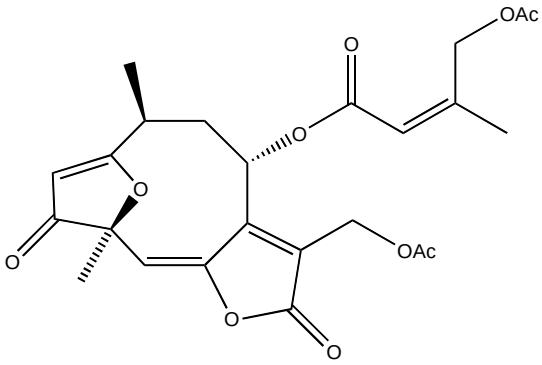

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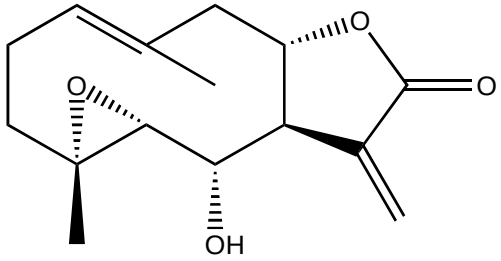

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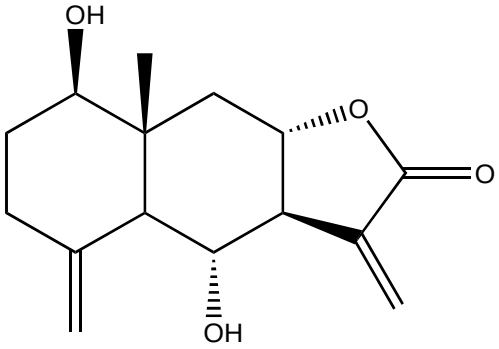

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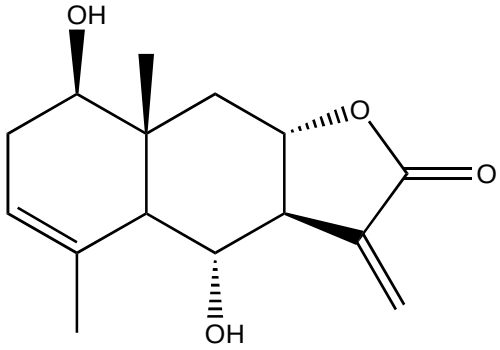
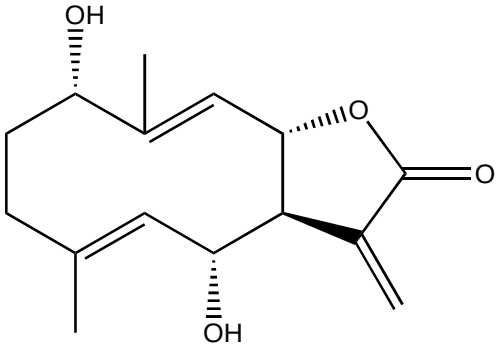
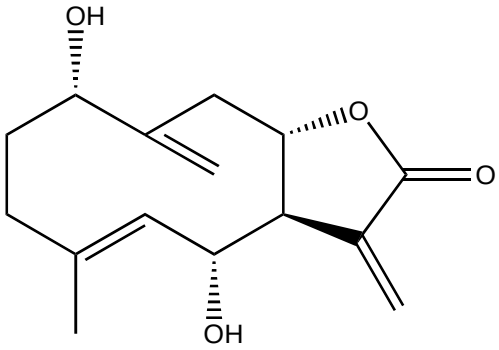


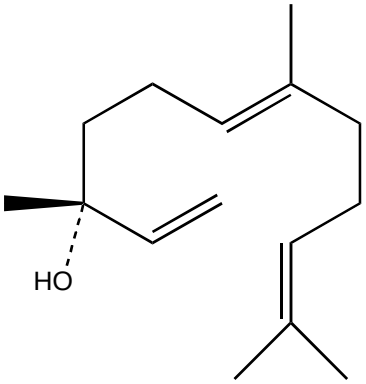
Sivasinolide **(8)**



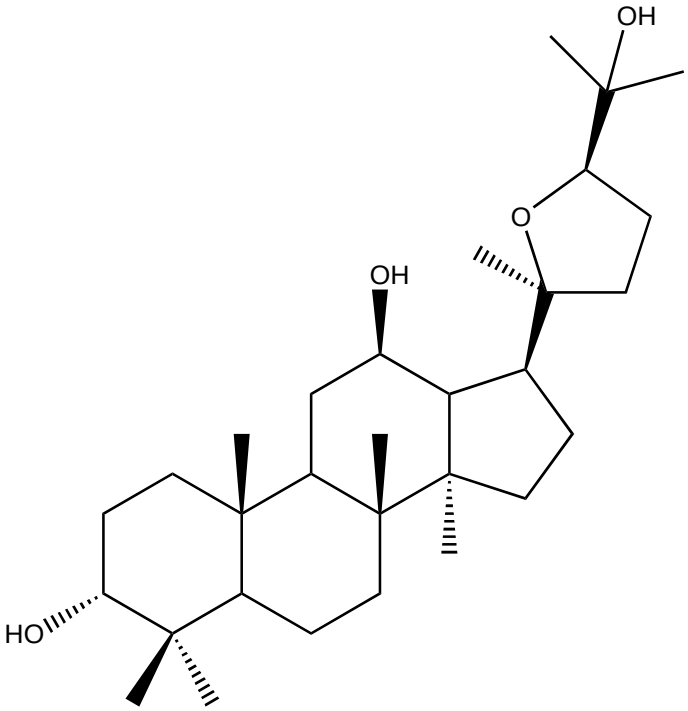
Tatridin A **(9)**



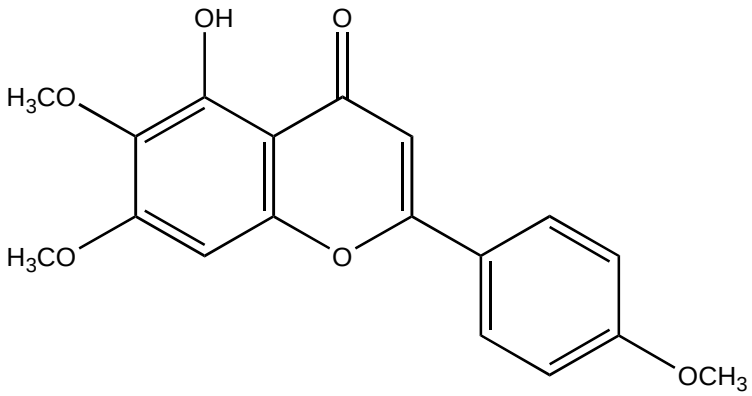
Tanachin **(10)**



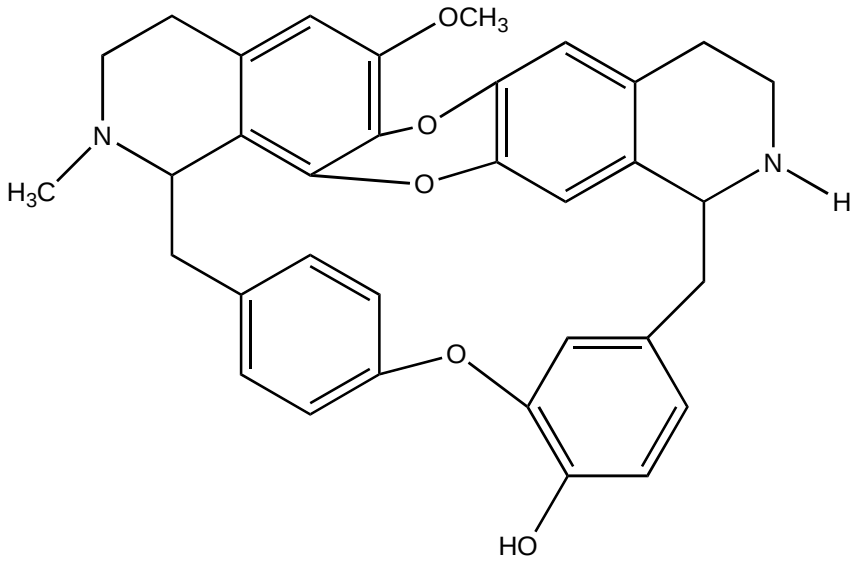
Nerolidol **(11)**



Betulafolientriol oxide **(12)**



Salvigenin **(13)**



Cocsoline **(14)**

Fig. 1. Structures of compounds **1** - 14