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Artemisia spp. & Artemisinin.
Abdin M.Z., Israr M., Rehman R.U. & Jain S.K. (2003) "Artemisinin, a novel antimalarial drug: biochemical and molecular approaches for enhanced production." *Planta Med.* 69(4), 289-99. Abstract. Artemisinin, a sesquiterpene lactone containing an endoperoxide bridge, has been isolated from the aerial parts of *Artemisia annua* L. plants. It is effective against both drug-resistant and cerebral malaria-causing strains of *Plasmodium falciparum*. The relatively low yield (0.01-0.8 %) of artemisinin in *A. annua* is a serious limitation to the commercialization of the drug. Therefore, the enhanced production of artemisinin either in cell/tissue culture or in the whole plant of *A. annua* is highly desirable. It can be achieved by a better understanding of the biochemical pathway leading to the synthesis of artemisinin and its regulation by both exogenous and endogenous factors. Furthermore, genetic engineering tools can be employed to overexpress gene(s) coding for enzyme(s) associated with the rate limiting step(s) of artemisinin biosynthesis or to inhibit the enzyme(s) of other pathway competing for its precursors. These aspects which may be employed to enhance the yield of artemisinin both in vitro and in vivo are discussed in this review.


van Agtmael M.A., Eggelte T.A., van Boxtel C.J.. (1999) "Artemisinin drugs in the treatment of malaria: from medicinal herb to registered medication." Trends Pharmacol Sci. 20(5), 199-205. Abstract. Registration in Europe of several artemisinin drugs for the treatment of malaria can soon be expected. Artemisinin is isolated from the herb Artemisia annua, in use in China more than 2000 years as a herbal tea against fever. Artemisinin drugs are being used extensively in South-East Asia and increasingly in Africa. Active derivatives have been synthesized - artemether, arteether and artesunate - which are used for oral, intramuscular, rectal and intravenous administration. The origin, mechanism of action, efficacy and safety in patients, the pharmacokinetics and the position of this group of compounds among existing antimalarials are discussed in this review.


Amos S., Chindo B.A., Abbah J., Vongtau H.O., Edmond I., Binda L., Akah P.A., Wambebe C., Gamaniel K.S. (2003) "Postsynaptic dopamine (D(2))-mediated behavioural effects of high acute doses of artemisinin in rodents." Brain Res Bull. 30, 62(3), 255-60. Abstract. Artemisinin or qinghaosu is the active principle of quinghao (Artemisia annua L.) developed from Chinese traditional medicine, which is now widely used around the world against falciparum malaria. Behavioural effects of high acute doses of artemisinin were studied on spontaneous motor activity (SMA), exploratory behavior, apomorphine-induced stereotype behavior and pentobarbital sleeping time in mice and rats in order to provide additional evidence on its safety profile on the central nervous system (CNS). Effects of the drug on bromocriptine-induced hyperactivity in short term reserpinised mice were also evaluated. Intraperitoneal (i.p.) injection of artemisinin at doses of 50 and 100mg/kg, significantly (P<0.05) reduced the SMA in mice, prolonged the pentobarbital sleeping time in rats, and attenuated the apomorphine-induced stereotypy in mice. Mice pretreated with reserpine, showed a significant decrease in locomotor activity compared to the saline-treated group. Bromocriptine, a D(2) receptor agonist, induced locomotor activity in mice pretreated with reserpine which was attenuated by artemisinin. The results suggest that artemisinin possesses sedative property, which may be mediated via postsynaptic dopamine (D(2)) receptor in the CNS.

Artemisinin (1) is a unique sesquiterpene peroxide occurring as a constituent of Artemisia annua L. Because of the effectiveness of Artemisinin in the treatment of drug-resistant Plasmodium falciparum and its rapid clearance of cerebral malaria, development of clinically useful semisynthetic drugs for severe and complicated malaria (artemether, artesunate) was prompt. However, recent reports of fatal neurotoxicity in animals with dihydroartemisinin derivatives such as artemether have spawned a renewed effort to develop nontoxic analogues of artemisinin. In our effort to develop more potent, less neurotoxic agents for the oral treatment of drug-resistant malaria, we utilized comparative molecular field analysis (CoMFA) and hologram QSAR (HQSAR), beginning with a series of 211 artemisinin analogues with known in vitro antimalarial activity. CoMFA models were based on two conformational hypotheses: (a) that the X-ray structure of artemisinin represents the bioactive shape of the molecule or (b) that the hemin-docked conformation is the bioactive form of the drug. In addition, we examined the effect of inclusion or exclusion of racemates in the partial least squares (pls) analysis. Databases derived from the original 211 were split into chiral (n = 157), achiral (n = 34), and mixed databases (n = 191) after leaving out a test set of 20 compounds. HQSAR and CoMFA models were compared in terms of their potential to generate robust QSAR models. The r(2) and q(2) (cross-validated r(2)) were used to assess the statistical quality of our models. Another statistical parameter, the ratio of the standard error to the activity range (s/AR), was also generated. CoMFA and HQSAR models were developed having statistically excellent properties, which also possessed good predictive ability for test set compounds. The best model was obtained when racemates were excluded from QSAR analysis. Thus, CoMFA of the n = 157 database gave excellent predictions with outstanding statistical properties. HQSAR did an outstanding job in statistical analysis and also handled predictions well.


Balint G.A. (2001) "Artemisinin and its derivatives: an important new class of antimalarial agents." Pharmacol Ther. 90(2-3), 261-5. Abstract. Artemisinin and its derivatives are a potent new class of antimalarials, originated from Artemisia annua, L. The clinical efficacy of these drugs is characterized by an almost immediate onset and rapid reduction of parasitaemia. Their efficacy is high in such areas as well where multidrug-resistance is rampant, but in these areas, their combination with other (effective) antimalarials (e.g., mefloquine) is highly
recommended. In this short review, the chemical structures, pharmacological properties, and clinical uses of artemisinin drugs are discussed.

Bertea C.M., Voster A., Verstappen F.W., Maffei M., Beekwilder J., Bouwmeester H.J. (2006) "Isoprenoid biosynthesis in *Artemisia annua*: cloning and heterologous expression of a germacrene A synthase from a glandular trichome cDNA library." *Arch Biochem Biophys.* **448**(1-2), 3-12. Abstract. *Artemisia annua* (Asteraceae) is the source of the anti-malarial compound artemisinin. To elucidate the biosynthetic pathway and to isolate and characterize genes involved in the biosynthesis of terpenoids including artemisinin in *A. annua*, glandular trichomes were used as an enriched source for biochemical and molecular biological studies. The sequencing of 900 randomly selected clones from a glandular trichome plasmid cDNA library revealed the presence of many ESTs involved in isoprenoid biosynthesis such as enzymes from the methylerithritol phosphate pathway and the mevalonate pathway, amorpha-4,11-diene synthase and other sesquiterpene synthases, monoterpene synthases and two cDNAs showing high similarity to germacrene A synthases. Full-length sequencing of the latter two ESTs resulted in a 1686-bp ORF encoding a protein of 562 aa. Upon expression in *Escherichia coli*, the recombinant protein was inactive with geranyl diphosphate, but catalyzed the cyclization of farnesyl diphosphate to germacrene A. These results demonstrate the potential of the use of *A. annua* glandular trichomes as a starting material for studying isoprenoid biosynthesis in this plant species.

Bilia A.R., Lazari D., Messori L., Taglioli V., Temperini C., Vincieri F.F. (2002) "Simple and rapid physico-chemical methods to examine action of antimalarial drugs with hemin: its application to *Artemisia annua* constituents." *Life Sci.* 2002 **4**, 70(7), 769-78. Abstract. Malaria is a major health problem in many countries and according to an estimate of the WHO, more than 500 million infections occur per year. Artemisinin, a sesquiterpene from *Artemisia annua* L., has received considerable attention as a promising and potent antimalarial drug for its stage specificity, its rather low toxicity, effectiveness against drug-resistant *Plasmodium* species and activity against cerebral malaria. From recent studies it seems that hemin is primarily involved in the antimalarial activity of the constituents of *Artemisia annua* L. Thus, the interaction of a compound with hemin may represent a crucial screening test to define its efficacy. In this study the interaction between artemisinin and hemin was investigated by UltraViolet/Visible (UV/Vis) spectrophotometry and High Performance Liquid Chromatography/Diode Array Detector/Mass Spectrometry (HPLC/DAD/MS). In addition, some flavonols isolated from *Artemisia annua* L. were also tested to investigate their possible role in the interaction between artemisinin and hemin. These two simple physico-chemical methods can be useful as rapid and widespread screening methods for the search of other alkylating antimalarial constituents from natural sources or for the evaluation of the activity of semisynthetic analogues of artemisinin.


Chawira A.N., Warhurst D.C., Robinson B.L., Peters W. (1987) "The effect of combinations of qinghaosu (artemisinin) with standard antimalarial drugs in the suppressive treatment of malaria in mice." *Trans R. Soc. Trop. Med. Hyg.* 81(4), 554-8. **Abstract.** Artemisinin is a novel antimalarial drug isolated in China from the wormwood plant *Artemisia annua* L. Studies with rodent malaria were carried out to detect antagonism and synergism with a variety of antimalarial drugs. Isobolograms of drug interaction were plotted at the ED90 level. With a normally susceptible strain of *Plasmodium berghei*, marked potentiative synergism was found with mefloquine, tetracycline and spiramycin. There was some synergism also with primaquine. Combinations of artemisinin with dapsone, sulfadiazine, sulfadoxine, pyrimethamine, pyrimethamine/sulfadoxine and cycloguanil showed antagonism. A high degree of potentiation was shown between artemisinin and primaquine with a primaquine-resistant strain, whilst the combination with mefloquine showed enhanced potentiation with a mefloquine-resistant strain. Combinations of artemisinin with mefloquine, primaquine, tetracycline or clindamycin showed marked potentiation with an artemisinin-resistant strain. The mechanisms underlying the drug interactions observed are discussed.

Cheng F., Shen J., Luo X., Zhu W., Gu J., Ji R., Jiang H. & Chen K. (2002) "Molecular docking and 3-D-QSAR studies on the possible antimalarial mechanism of artemisinin analogues." *Bioorg Med Chem.* 10(9), 2883-91. **Abstract.** Artemisinin (Qinghaosu) is a natural constituent found in *Artemisia annua* L, which is an effective drug against chloroquine-resistant *Plasmodium falciparum* strains and cerebral malaria. The antimalarial activities of artemisinin and its analogues appear to be mediated by the interactions of the drugs with hemin. In order to understand the antimalarial mechanism and the relationship between the physicochemical properties and the antimalarial activities of artemisinin analogues, we performed molecular docking simulations to probe the interactions of these analogues with hemin, and then performed three-dimensional quantitative structure-activity relationship (3-D-QSAR) studies on the basis of the docking models employing comparative molecular force fields analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). Molecular docking simulations generated probable 'bioactive' conformations of artemisinin analogues and provided a new insight into the antimalarial mechanism. The subsequent partial least squares (PLS) analysis
indicates that the calculate binding energies correlate well with the experimental activity values. The CoMFA and CoMSIA models based on the bioactive conformations proved to have good predictive ability and in turn match well with the docking result, which further testified the reliability of the docking model. Combining these results, that is molecular docking and 3-D-QSAR, together, the binding model and activity of new synthesized artemisinin derivatives were well explained.


Delhaes L., Benoit-Vical F., Camus D., Capron M. & Meunier B. (2003) "Chloroquine and artemisinin: six decades of research--what next?" Drugs 6(7), 674-80. Abstract. Over the next decade drugs will remain the focus of continuous efforts to control malaria, with a contribution from pharmacogenomic development. Quinine, extracted from Cinchona bark, has been the source for aminoquinoline drugs such as chloroquine; more recently, artemisinin extracted from Artemisia allowed the design of artemisinin mimics containing a trioxane structure. Here, we examine parallels between chloroquine and artemisinin in terms of pharmacological target discovery, mechanism of action and parasite resistance. The widespread use of chloroquine has dramatically reduced its therapeutic response, thus recent strategies are based on artemisinin combinations.

Dobson M.J. (1998) “Bitter-sweet solutions for malaria: exploring natural remedies from the past.” Parassitologia 40(1-2), 69-81. Abstract. This paper explores "a wonderful cure" for malaria used successfully by Robert Talbor, an apothecary's apprentice in the English marshes, to treat Essex smugglers and European Royalty in the seventeenth century. The basis of this cure is identified as "quinquina" from the bark of the South American Cinchona tree. The story of Robert Talbor and his secret remedy for malaria opens up a set of intriguing questions about the early history of "quinquina", the subsequent development of quinine, the use of higher plants for antimalarial drugs, including the Chinese plant Artemisia annua L., and the value of unlocking the secrets of the past in our search for strategies to control malaria.


Artemisinins are extracted from sweet wormwood (*Artemisia annua*) and are the most potent antimalarials available, rapidly killing all asexual stages of *Plasmodium falciparum*. Artemisinins are sesquiterpene lactones widely used to treat multidrug-resistant malaria, a disease that annually claims 1 million lives. Despite extensive clinical and laboratory experience their molecular target is not yet identified. Activated artemisinins form adducts with a variety of biological macromolecules, including haem, translationally controlled tumour protein (TCTP) and other higher-molecular-weight proteins. Here we show that artemisinins, but not quinine or chloroquine, inhibit the SERCA orthologue (PfATP6) of *Plasmodium falciparum* in Xenopus oocytes with similar potency to thapsigargin (another sesquiterpene lactone and highly specific SERCA inhibitor). As predicted, thapsigargin also antagonizes the parasiticidal activity of artemisinin. Desoxyartemisinin lacks an endoperoxide bridge and is ineffective both as an inhibitor of PfATP6 and as an antimalarial. Chelation of iron by desferrioxamine abrogates the antiparasitic activity of artemisinins and correspondingly attenuates inhibition of PfATP6. Imaging of parasites with BODIPY-thapsigargin labels the cytosolic compartment and is competed by artemisinin. Fluorescent artemisinin labels parasites similarly and irreversibly in an Fe$^{2+}$-dependent manner. These data provide compelling evidence that artemisinins act by inhibiting PfATP6 outside the food vacuole after activation by iron.


Abstract. Artesunate (ART) is a semi-synthetic derivative of artemisinin, the active principle of the Chinese herb *Artemisia annua*. ART reveals remarkable activity against otherwise multidrug-resistant *Plasmodium falciparum* and *P. vivax* malaria. ART has now been analyzed for its anti-cancer activity against 55 cell lines of the Developmental Therapeutics Program of the National Cancer Institute, USA. ART was most active against leukemia and colon cancer cell lines (mean GI50 values: 1.11+/-0.56 microM and 2.13+/-0.74 microM, respectively). Non-small cell lung cancer cell lines showed the highest mean GI50 value (25.62+/-14.95 microM) indicating the lowest sensitivity towards ART in this test panel. Intermediate GI50 values were obtained for melanomas, breast, ovarian, prostate, CNS, and renal cancer cell lines. Importantly, a comparison of ART's cytotoxicity with those of other standard cytostatic drugs showed that ART was active in molar ranges comparable to those of established anti-tumor drugs. Furthermore, we tested CEM leukemia sub-lines resistant to either doxorubicin, vincristine, methotrexate, or hydroxyurea which do not belong to the N.C.I. screening panel. None of these drug-resistant cell lines showed cross resistance to ART. To gain insight into the molecular mechanisms of ART's cytotoxicity, we used a panel of isogenic *Saccaromyces cerevisiae* strains with defined genetic mutations in DNA repair, DNA checkpoint and cell proliferation genes. A yeast strain with a defective mitosis regulating BUB3 gene showed increased ART sensitivity and another strain with a defective proliferation-regulating CLN2 gene showed increased ART resistance over the wild-type strain, wt644. None of the other DNA repair or DNA check-point deficient isogenic strains were different
from the wild-type. These results and the known low toxicity of ART are clues that ART may be a promising novel candidate for cancer chemotherapy.

Elford B.C., Roberts M.F., Phillipson J.D. & Wilson R.J. (1987) "Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones." Trans R Soc Trop Med Hyg. 81(3), 434-6 Abstract. Interaction between the flavones casticin and artemetin and the antimalarial activity of chloroquine and qinghaosu (QHS) was examined using an in vitro growth assay based on [3H]hypoxanthine incorporation in synchronized cultures of a cloned line of *Plasmodium falciparum*. Casticin, and to a lesser extent artemetin, selectively enhanced the inhibition of growth by QHS, but had little effect on the activity of chloroquine. The findings suggest that flavones indigenous to Artemisia annua, from which QHS is isolated, might significantly alter the clinical potential of this novel antimalarial drug in the treatment of chloroquine-resistant malaria.


Van Geldre E., Vergauwe A. & Van den Eeckhout E. (1997) "State of the art of the production of the antimalarial compound artemisinin in plants." Plant Molecular Biology 33: 199–209, 1997. Abstract. For more than three centuries we have relied on the extracts of the bark of Cinchona species to treat malaria. Now, it seems we may be changing to the leaves of a Chinese weed, *Artemisia annua*, and its active compound artemisinin. Artemisinin-derived drugs have been proved particularly effective treatments for severe malaria, even for multidrug-resistant malaria. However, this promising antimalarial compound remains expensive and is hardly available on a global scale. Therefore, many research groups have directed their investigations toward the enhancement of artemisinin production in *A. annua* cell cultures or whole plants in order to overproduce artemisinin or one of its precursors. This article provides a brief review of the state of art of the different aspects in *A. annua* research.

Guo X.B. & Fu L.C. (1989) “Comparitive study of artemisinin suppositories & piperaquin phosphate in the treatment of falciparum malaria.” Zhong Xi Yi Jie He Za Zhi 9(8), 475-477, 483. Abstract. Artemisinin, developed by Chinese scientists, is a new type of anti-malarial drug with quick effect and low toxicity. Since its solubility in water or oil is very low, it cannot be made into a clear injection to be given intramuscularly or intravenously for emergency use. The artemisinin suppositories used in the study was provided by the institute of Chinese Materia Medica in 1982. Phase I and Phase II clinical trials of the drug were made by Guangzhou College of TCM. The results showed that the therapeutic effect of Artemisinin suppositories was satisfactory with no apparent side effects. The total dosage recommended was 2800-3200 mg. In 1986, fifty-six adults with falciparum malaria were treated with a total dose of 2800 mg Artemisinin suppositories for 3 days and randomly compared with a control group of Piperaquine phosphate in the Dongfang Town Hospital, Dongfang ( ) County
of Hainan Island. The parasite clearance time in Artemisinin suppositories group (71.8 +/- 16.0 hrs) was significantly faster than that of Piperaquine phosphate group (100.3 +/- 20.3hrs), but recrudescence rate by 28 days (48.2%) was much higher than that of Piperaquine phosphate (17.0%). Artemisinin suppositories is simple to administrate and therefore it could be applied in endemic area of remote countryside and to the patients of incapable of oral dosing.

Haynes R.K. (2006). “From artemisinin to new artemisinin antimalarials: biosynthesis, extraction, old and new derivatives, stereochemistry and medicinal chemistry requirements” Curr Top Med Chem. 6(5), 509-37. Abstract. The artemisinin derivatives, dihydroartemisinin (DHA), artesunate, atemether and arteether, are currently used for treatment of malaria in artemisinin combination therapies (ACT) with longer half-life drugs. The demand is enormous—in 2005, the estimated global demand for one such ACT alone, arteether-lumifantrine, which constitutes about 70% of all current clinically-used ACTs, is for 120 million adult treatment courses. At 0.5 gm of arteether per total dose regimen, the amount of artemisinin required is approximately 114 tons. This has placed substantial stress on total artemisinin supplies world-wide, and considerable attention is being focussed on enhancing availability of artemisinin by improvement in horticultural practice and extraction of artemisinin from Artemisia annua. Artemisinic acid, which also occurs in A. annua, can be converted into artemisinin and is the ultimate target of a biotechnological approach, which if successful, will augment artemisinin supply in the future. The conversion of artemisinin into the known artemisinin derivatives, and problems with the methods are critically reviewed. Some attention is paid to mechanistic aspects which clarify stereochemistry. The current artemisinins are by no means ideal drugs. Artesunate in particular is incompatible with basic quinolines by virtue of proton transfer, and has intrinsic chemical instability. At pH 1.2, conversion to DHA is rapid, with t(1/2) 26 min, and at pH 7.4, t(1/2) is about 10 hours. With a pK(a) of 4.6, over 99% of artemisinin will be ionized at pH 7.4, and thus uptake by passive diffusion from the intestinal tract will be minimal. Although a considerable effort has been vested in the search for new artemisinins, largely through functionalization of arteether at C-10, O-11 or at C-15 via arteisitene, or of DHA at C-10, deliberate enhancement of the 'druggability' of artemisinins by reducing lipophilicity, which at the same time will attenuate the neurotoxicity characteristic of the current derivatives, and enhance absorption, by and large has not been considered. A review of the various types of newer derivatives is given together with a consideration of medicinal chemistry aspects.

Hsu E. (2006) “The history of qing hao in the Chinese materia medica.” Trans R Soc Trop Med Hyg. 100(6):505-8. Abstract. Artemisinin is currently used for treating drug-resistant malaria. It is found in Artemisia annua and also in A. apiacea and A. lancea. Artemisia annua and A. apiacea were known to the Chinese in antiquity and, since they were easily confused with each other, both provided plant material for the herbal drug qing hao (blue-green hao). This article shows, however, that since at least the eleventh century Chinese scholars recognized the difference between the two species, and advocated the use of A.
*Artemisia apiacea*, rather than *A. annua* for 'treating lingering heat in joints and bones' and 'exhaustion due to heat/fevers'. The article furthermore provides a literal translation of the method of preparing qing hao for treating intermittent fever episodes, as advocated by the eminent physician Ge Hong in the fourth century CE. His recommendation was to soak the fresh plant in cold water, wring it out and ingest the expressed juice in its raw state. Both findings may have important practical implications for current traditional usage of the plant as an antimalarial: rather than using the dried leaves of *A. annua* in warm infusions, it suggests that fresh juice extraction from *A. apiacea* may improve efficacy.


Klayman D.L. (1985) "Qinghaosu (artemisinin): an antimalarial drug from China." Science. 31, 228(4703), 1049-55. Abstract. The herb *Artemisia annua* has been used for many centuries in Chinese traditional medicine as a treatment for fever and malaria. In 1971, Chinese chemists isolated from the leafy portions of the plant the substance responsible for its reputed medicinal action. This compound, called qinghaosu (QHS, artemisinin), is a sesquiterpene lactone that bears a peroxide grouping and, unlike most other antimalarials, lacks a nitrogen-containing heterocyclic ring system. The compound has been used successfully in several thousand malaria patients in China, including those with both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. Derivatives of QHS, such as dihydroqinghaosu, artemether, and the water-soluble sodium artesunate, appear to be more potent than QHS itself. Sodium artesunate acts rapidly in restoring to consciousness comatose patients with cerebral malaria. Thus QHS and its derivatives offer promise as a totally new class of antimalarials.

Kurtzhals J.A. (2005) ."Ineffective change of antimalaria prophylaxis to *Artemisia vulgaris* in a group travelling to West Africa".Ugeskr Laeger. 24, 167(43), 4082-3. Abstract. Sixteen travellers to West Africa used four kinds of antimalaria chemoprophylaxis. Suspected malaria in three persons and vaginal candidiasis in one caused all seven doxycycline users to change their medication. One of these was persuaded to use *Artemisia vulgaris* extract. In the course of the three-month journey, there were seven suspected cases of malaria, only two of which could be confirmed by antibody and antigen detection or expert microscopy; both were in travellers who had used *A. vulgaris*. *A. vulgaris* had no effect on parasite growth in vitro. The use of natural products for malaria prophylaxis should be discouraged. Cropwatch Comments. "The use of natural products for malaria prophylaxis should be discouraged" seems a little harsh. Whilst not defending the sole use of *Artemisia vulgaris* in this scenario, see Hwang Y–S. (1985) “Isolation & identification of mosquito repellents from *Artemisia vulgaris*. J. Chem. Ecol. 51, 1297-1306.

Lee M.R. (2002) "Plants against malaria, part 2: *Artemisia annua* (Qinghaosu or the sweet wormwood)." J.R. Coll Physicians Edinb. 32(4), 300-5
Li Y. & Wu Y.L. (1998) “How Chinese scientists discovered qinghaosu (artemisinin) and developed its derivatives? What are the future perspectives?” Med Trop (Mars) 58(3 Suppl):9-12. Abstract. Since the middle of this century and especially since the 1960s and 1970s. Chinese scientists have put considerable effort and resources into the search for new antimalarial compounds extracted from Chinese traditional herbs. Archaeological findings indicate that qinghao (Artemisia annua L.) has been used as a traditional remedy in China for over two thousand years. Its antimalarial principle was finally isolated in 1971 and named artemisinin or qinghaosu (meaning the principle of qinghao in Chinese). Its rapid action, low toxicity and powerful effect against falciparum malaria made it a favored subject for research. In 1976, the unique structure of the molecule, characterized by an endoperoxide and an alternative O-C-O-C segment, was identified. The specific lactone reduction discovered during the determination of the structure opened the way for the synthesis of qinghaosu derivatives, and thereafter a series of more active and more oil- or water-soluble derivatives was developed. Subsequent studies of the structure/activity relationship led to the discovery of dihydroartemisinin, artemether and artesunate. Now qinghaosu and these three derivatives are being used around the world as effective new antimalarial drugs in the fight against falciparum malaria, including multi-drug-resistant Plasmodium falciparum. At the present time new qinghaosu analogues or derivatives are being developed and studies of their structure/activity relationships, their antimalarial mechanisms, their interaction with ferrous ions and the DNA damage associated with these processes are being actively pursued. In addition, recent studies also indicate that some qinghaosu derivatives have other bioactivities, including antiparasitic (against Schistosoma japonicum, Toxoplasma gondii and so on) and anticancer activities. Research into qinghaosu and its derivatives has already produced and will no doubt continue to produce results of the utmost importance in the fight against malaria and other diseases.

Liu A.R., Yu Z.Y., Lu L.L. & Sui Z.Y. (2000) “The synergistic action of guanghuoxiang volatile oil and sodium artesunate against Plasmodium berghei and reversal of SA-resistant Plasmodium berghei.” Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 18(2),76-8. Abstract. OBJECTIVE: To study the synergistic action of a combination of guanghuoxiang volatile oil (B) and sodium artesunate (SA) against Plasmodium berghei (P. b) and the resistance-reversal activity against SA-resistant P. b (P. b SA-R). METHODS: Mice infected with P. b N or P. b R were treated with a combination of B and SA respectively by 4-day suppressive test method and linear regression to calculate the SD50 of B and SA for each drug alone and in combination (equally effective dose compatibility). RESULTS: B alone, N:SD50 = 87.64 +/- 19.58(GKD), R:SD50 = 43.24 +/- 7.71(GKD); SA alone, N:SD50 = 0.88 +/- 0.01(MGKD), R:SD50 = 27.69 +/- 0.93(MGKD). B and SA combination, N:B SD50 = 36.89 +/- 4.57(GKD), SA SD50 = 0.39 +/- 0.05 (MGKD); R:B SD50 = 7.40 +/- 1.30(GKD), SA SD50 = 4.21 +/- 0.74(MGKD). The synergistic indexes of B and SA in combination were 2.2 for N and 6.6 for R, respectivly. The multiple of resistance reversal of B vs SA was 6.6. The relative reversal rate was 87.6%. CONCLUSION: A combination of B
and SA may enhance the antimalarial effect against P. b and reverse the SA-resistance of P. b and delay the occurrence of resistance to SA in N.

Liu C., Zhao Y. & Wang Y. (2006). “Artemisinin: the current state and perspectives for the biotechnological production of an anti-malarial drug.” Applied Microbiol. Biotech. 72(1), 11-20. Abstract: Artemisinin isolated from the aerial parts of Artemisia annua L. is a promising and potent antimalarial drug which has a remarkable activity against chloroquine-resistant and chloroquine-sensitive strains of Plasmodium falciparum, and is useful in treatment of cerebral malaria. Because the low content (0.01-1 %) of artemisinin in A. annua is a limitation to the commercial production of the drug, many research groups have been focusing their researches on enhancing the production of artemisinin in tissue culture or in the whole plant of A. annua. This review mainly focuses on the progresses made in the production of artemisinin from A. annua by biotechnological strategies including in vitro tissue culture, metabolic regulation of artemisinin biosynthesis, genetic engineering, and bioreactor technology.


Ma C., Wang H., Lu X., Li H., Liu B. & Xu G. (2007) "Analysis of Artemisia annua L. volatile oil by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry." J. Chromatogr A. 1150(1-2), 50-3. Abstract. Artemisia annua L. is an annual herb native of Asia, it has been used for many centuries for the treatment of fever and malaria. In this paper, analysis of the volatile oil of Artemisia annua L. was performed by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC x GC-TOF MS). Three hundred and three components were tentatively identified and terpene compounds are the main components of Artemisia annua L. volatile oil. Artemisinic acid is tentatively qualified.

Malagon F, Vazquez J, Delgado G, Ruiz A. (1997) "Antimalaric effect of an alcoholic extract of Artemisia ludoviciana mexicana in a rodent malaria model." Parassitologia 39(1), 3-7 Abstract. Chloroquine resistance of Plasmodium falciparum first and of P. vivax more recently, stimulated the search for new antimalarics. Chinese investigators have introduced new compounds obtained from extracts of Artemisia annua which possess an antimalaric active principle different from those of the drugs in use. In Mexico eight species of Artemisia have been described and among them just A. ludoviciana has been empirically used in the treatment of intermittent fever. To know whether mexican Artemisia had antimalaric activity several in vivo experiments were performed. Different type of extracts from two Artemisia species were prepared and assayed in five different doses on mice infected by Plasmodium yoelii yoelii, in a four-day test scheme. Here, only the results of the assays on ethanolic extract of A. ludoviciana are presented. The results of the in vivo experiments showed that the
parasite reproduction was inhibited up to 98.6% at the fifth day, as compared with the controls; the ED50 was of 29.2 mg/kg and the SM50 of 28.7. We looked after the presence of artemisinin in the ethanolic extract, without success.


van der Meersch H. (2005). “Review of the use of artemisinin and its derivatives in the treatment of malaria”. J Pharm Belg. 60(1), 23-9. Abstract. This article reviews the development of the artemisinins used in the treatment of drug-resistant Plasmodium falciparum malaria. The story starts in China with Artemisia annua L., a plant that was traditionally used as an antipyretic. The activity of Annual wormwood can be explained by the presence of the active substance artemisinin. Soon, artemether, artremotil, artemimol, artesunate and sodium artesunate, derivatives of artemisinin, have been developed. Each has its own physical and pharmaceutical properties, dosage and dosage forms. Other aspects, such as the general guidelines for use, safety during pregnancy and the perspectives of artemisinin compounds, are being discussed.

Mesia G.K., Tona G.L., Penge O., Lusakibanza M., Nanga T.M., Cimanga R.K., Apers S., Van Miert S., Totte J., Pieters L. & Vlieghe H. (2005) "Antimalarial activities and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo: Croton mubango, Nauclea pobeguinii and Pyrenacantha staudtii." Ann Trop Med Parasitol. 99(4), 345-57. Abstract. The antimalarial activities of crude extracts and 17 fractions from the partition of 80%-methanolic extracts of three plants (the stem bark of Croton mubango, the stem bark of Nauclea pobeguinii and the leaves of Pyrenacantha staudtii) used as antimalarial remedies in the Democratic Republic of Congo were studied both in vitro (against Plasmodium falciparum) and in mice infected with Pl. berghei berghei. The toxic effects of dried aqueous extracts of the plants were also investigated, in uninfected mice. The most active crude extracts in vitro, with median inhibitory concentrations (IC(50)) of <1 microg/ml, were found to be the methanolic and dichloromethane extracts of C. mubango, and the dichloromethane extracts of N. pobeguinii and Py. staudtii. The aqueous extract with the most antimalarial activity in vitro was that of C. mubango (IC(50) = 3.2 microg/ml), followed by that of N. nobeguinii (IC(50) = 5.3 microg/ml) and then that of Py. staudtii (IC(50) = 15.2 microg/ml). Results from the in-vivo tests of antimalarial activity showed that, at a daily oral dose of 200 mg/kg, all the dichloromethane extracts, the petroleum-ether, chloroformic, ethyl-acetate and residual water-soluble fractions from C. mubango, and the chloroformic, ethyl-acetate and n-butanolic fractions from Py. staudtii produced >80% chemosuppression of the parasitaemias by day 4. The aqueous extracts of C. mubango and N. nobeguinii produced a slightly lower but still significant inhibition of parasitaemia (60%-80%) whereas that of Py. staudtii only suppressed the day-4 parasitaemias by 37%.The dried aqueous extract of the stem bark of C. mubango showed some signs of toxicity in mice, with median lethal doses (LD(50)) of 350 mg/kg in the female mice and 900 mg/kg in the
male. The extract significantly increased the serum concentrations of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) in mice of both sexes, but had no effect on the blood levels of creatinine or urea. No significant toxic effect was observed for the dried aqueous extracts of N. pobeguinii and Py. staubtii (LD(50) >5 g/kg). Neither of these extracts affected the serum concentrations of GPT or the blood concentrations of creatinine and urea, although the N. pobeguinii extract did increase the serum concentration of GOT.

Mueller M.S., Runyambo N., Wagner I., Borrmann S., Dietz K. & Heide L. (2004) "Randomized controlled trial of a traditional preparation of Artemisia annua L. (Annual Wormwood) in the treatment of malaria." Trans R Soc Trop Med Hyg. 98(5), 318-21. Abstract. The Chinese medicinal plant Artemisia annua L. (Annual Wormwood) contains the antimalarial compound artemisinin. The locally grown herb may offer an additional tool for the control of malaria, especially in poor countries where modern antimalarial drugs are often unavailable. In an open, randomized, controlled pilot trial, we investigated the efficacy and safety of traditional tea preparations of Artemisia annua in the treatment of uncomplicated malaria. Treatment resulted in a quick resolution of parasitaemia and of clinical symptoms. After 7 d of medication, cure rates were on average 74% for the Artemisia preparations compared with 91% for quinine. However, recrudescence rates were high in the Artemisia groups. Therefore, monotherapy with Artemisia annua L. cannot be recommended as alternative to modern antimalarials, but may deserve further investigation.

Niu X.Y., Ho L.Y., Ren Z.H. & Song Z.Y. (1985) "Metabolic fate of Qinghaosu in rats; a new TLC densitometric method for its determination in biological material." Eur J. Drug Metab Pharmacokinet. 10(1), 55-9. Abstract. Since the sixties, the emergence of malarial parasites resistant to the most potent anti-malarials has posed a serious problem to the therapy of malaria. Qinghaosu, a new sesquiterpene isolated from a Chinese medicinal herb Qing-hao (Artemisia annua Linn) is being used for the treatment of malaria in China with good results even in cases resistant to common anti-malarial agents. In this paper, a sensitive method of high specificity using TLC for the determination of Qinghaosu in biological specimens and in the study of the metabolism of the drug in rats is described. Qinghaosu was shown to be completely and rapidly absorbed after oral administration. However, a very low plasma level was obtained even after a dose of 300 mg/kg. Liver was found to be the chief site of its inactivation. When Qinghaisu was given intramuscularly, significant and more persistent plasma levels were detected. Qinghaosu was shown to pass the blood-brain and blood-placenta barriers after i.v. injection. Very little unchanged Qinghaosu was found in the urine and feces in 48 hours regardless of administration route (i.v., i.m. or p.o.).

Transformation of the anti-malarial drug artemisinin by the fungi *Eurotium amstelodami* and *Aspergillus niger* were investigated. Cultures were grown in sucrose/malt broth with artemisinin for 14 days and extracted with ethyl acetate. Extracts were characterized by liquid chromatography. Two metabolites from each fungal extract were isolated and identified using mass spectrometry and nuclear magnetic resonance. 5-Beta-hydroxyartemisinin and 7-beta-hydroxyartemisinin were isolated in 63 and 32% yields, respectively, from the extract of *E. amstelodami*, and 80 and 19%, respectively, from the extract of *A. niger*.

Peters W., Li Z.L., Robinson B.L., Warhurst D.C. (1986) "The chemotherapy of rodent malaria, XL. The action of artemisinin and related sesquiterpenes." *Ann Trop Med Parasitol.* 80(5), 483-9. Abstract. Artemisinin (Qinghaosu), a poorly soluble sesquiterpene lactone derived from the plant *Artemisia annua* Linn., and a number of more soluble, semi-synthetic derivatives are rapidly-acting blood schizontocides against *Plasmodium berghei* and *P. yoelii nigeriensis*. An oily suspension of artemisinin given s.c. is more effective than aqueous suspensions. The activity is retained against lines resistant to primaquine, cycloguanil, pyrimethamine, sulphonamides, mefloquine and menoctone, but a highly chloroquine-resistant line is much less sensitive. Artemisinin has no causal prophylactic, gametocytocidal or sporontocidal action. Dihydroartemisinin causes the pigment of *P. berghei* to clump, but in a different fashion from the pigment changes induced by chloroquine or quinine, reflecting a different mode of action of the sesquiterpenes from that of these other antimalarials.

Rath K., Taxis K., Walz G., Gleiter C.H., Li S.M. & Heide L. (2004) “Pharmacokinetic study of artemisinin after oral intake of a traditional preparation of *Artemisia annua* L. (annual wormwood).” Abstract. *Artemisia annua* L. (annual wormwood) contains the antimalarial artemisinin. Aqueous preparations of the dried herb are included in the pharmacopoeia of the People’s Republic of China for treatment of fever and malaria. Fourteen healthy male volunteers received one liter of tea prepared from nine grams of *Artemisia annua* leaves. Blood samples were taken and artemisinin was detected by reversed phase high-performance liquid chromatography. The mean +/- SD maximum plasma concentration of artemisinin was 240 +/- 75 ng/mL and the mean +/- SD area under the plasma concentration-time curve was 336 +/- 71 ng/mL x hr. Artemisinin was absorbed faster from herbal tea preparations than from oral solid dosage forms, but bioavailability was similar. One liter of an aqueous preparation of nine grams of *Artemisia annua* contained 94.5 milligrams of artemisinin (approximately 19% of the usually recommended daily dose). Artemisinin plasma concentrations after intake of this herbal tea are sufficient for clinical effects, but insufficient to recommend such preparations as equivalent substitutes for modern artemisinin drugs in malaria therapy.


**Abstract.** Malaria is a global health problem that threatens 300-500 million people and kills more than one million people annually. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*. Synthetic antimalarial drugs and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing. Artemisinin, a sesquiterpene lactone endoperoxide extracted from *Artemisia annua* L (family Asteraceae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant *Plasmodium* spp., but is in short supply and unaffordable to most malaria sufferers. Although total synthesis of artemisinin is difficult and costly, the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of artemisinin. Here we report the engineering of *Saccharomyces cerevisiae* to produce high titres (up to 100 mg l(−1)) of artemisinic acid using an engineered mevalonate pathway, amorpha-4,11-diene synthase, and a novel cytochrome P450 monooxygenase (CYP71AV1) from *A. annua* that performs a three-step oxidation of amorpha-4,11-diene to artemisinic acid. The synthesized artemisinic acid is transported out and retained on the outside of the engineered yeast, meaning that a simple and inexpensive purification process can be used to obtain the desired product. Although the engineered yeast is already capable of producing artemisinic acid at a significantly higher specific productivity than *A. annua*, yield optimization and industrial scale-up will be required to raise artemisinic acid production to a level high enough to reduce artemisinin combination therapies to significantly below their current prices.

Phan V.T. (2002) “Artemisinine and artesunate in the treatment of malaria in Vietnam (1984-1999)” *Bull Soc Pathol Exot.* 95(2), 86-8. **Abstract.** The long history of the use of *Artemisia annua* L. to treat malaria (called Quinghao in China and Thanh hao in Vietnam) has led Vietnamese scientists to manufacture locally preparations of artemisinine and artesunate, to test their tolerance for human beings as well as their efficiency in treating *P. falciparum* and *P. vivax* infections. Associating these drugs with antibiotics (such as tetracycline or doxycycline) could be an interesting topic for future research. Under the auspices of the National Program against Malaria, specialists will try to prevent the occurrence of drug resistance in *Plasmodium* and to propose new associations of drugs.

Quinghaosu Antimalarial Coordinating Research Group (1979) “Anti-malarial studies on Qinghaosu.” *Chin. Med. J.* 92, 811. **Abstract:** An effective anti-malarial constituent was extracted from a traditional Chinese medicinal herb – Quinghao (*Artemisia annua* L.) in 1972. It was named Qinghaosu. According to the data from spectral analysis, X-ray diffraction analysis and chemical reaction it is a new type sesquiterpene lactone with a per-oxy group. Pharmacologic studies and clinical observations in every type of malarial infection show that Qinghaosu is a new type malaria drug with rapid action and low toxicity. It has direct parasiticidal action on plasmodium in the erythrocytic stage. The parasites in
*Plasmodium vivax* and *Plasmodium falciparum* (including cerebral malaria & chloroquine-resistant falciparum malaria) especially in the areas of chloroquine-resistant falciparum malaria were cleared more rapidly than that with chloroquine & quinine etc. It is ineffective in the tissue stage. In general the short term recurrence rate is higher with Qinghaosu than with chloroquine.

Sharma P, Mohan L. & Srivastava CN. (2006). “Phytoextract-induced developmental deformities in malaria vector.” Bioresour Technol. 2006 97(14), 1599-604. **Abstract.** Larvicidal potential of petroleum ether (Pee), carbon tetrachloride (Cte) and methanol extract (Mee) of *Artemisia annua*, *Chenopodium album* and *Sonchus oleraceus* was observed against malaria vector, *Anopheles stephensi* Liston. The Pee of *A. annua* with LC50 16.85 ppm after 24 h and 11.45 ppm after 48 h of treatment was found most effective, followed by Cte of *A. annua* and *Ch. album*, Pee of *Ch. album* and Mee of *A. annua*. However, no significant larvicidal activity was observed in Mee of *Ch. album* and all the three extracts of *S. oleraceus*. The Pee of *A. annua* was further investigated for its effect on the metamorphosis and the development of the malaria vector. It influenced the early life cycle of *An. stephensi* by reducing the percentage of hatching, larval, pupal and adult emergence and also lengthening the larval and pupal periods. The growth index was also reduced significantly. As the extract has remarkable effect on the metamorphosis and high larvicidal potential, it could, therefore, be used as an effective biocontrol agent against the highly nuisance malaria vector.

Sriram D., Rao V.S., Chandrasekhara K.V., & Yogeeswari P. (2004) "Progress in the research of artemisinin and its analogues as antimalarials: an update." Nat Prod Res. 18(6), 503-27. **Abstract.** Malaria is the number one infectious disease in the world today. Worldwide, over two million people die each year from malaria. This shocking reality is largely due to the emergence of drug resistant strains of *Plasmodium falciparum*. Artemisinin, a sesquiterpene lactone endoperoxide isolated from *Artemesia* (sic) *annua* has been shown to be a fast acting, safe and effective drug against multidrug-resistant and sensitive strains of *P. falciparum*. This article reports a survey of the literature dealing with artemisinin related antimalarial issues that have appeared from 1980s to the beginning of 2003. A broad range of medical and pharmaceutical disciplines is covered, including a brief introduction about discovery, phytochemical aspects, antimalarial mechanism of action, pharmacokinetics, and major drawbacks and various structural modifications made to overcome them.

Tan RX, Zheng WF, Tang HQ (1998) "Biologically active substances from the genus *Artemisia.*" Planta Med. 64(4), 295-302. **Abstract.** Artemisia species, widespread in nature, are frequently utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses. Furthermore, some *Artemisia* constituents were found to be potential insecticides and allelopathic chemicals. This genus is receiving growing attention presumably due to: (i) the diversified biology and chemistry of the constituents, (ii) the frequent application in traditional medical practice, and (iii) the rich source
of the plant material. This review summarizes mainly the biological results obtained in the past decade. The significance and trends in this field are briefly discussed.

Tawfik A.F., Bishop S.J., Ayalp A. & el-Feraly F.S. (1990) "Effects of artemisinin, dihydroartemisinin and arteether on immune responses of normal mice." *Int J Immunopharmacol.* **12**(4), 385-9 [Abstract]. Artemisinin (Qinghaosu) is a potent antimalarial sesquiterpene lactone isolated from the Chinese herb *Artemisia annua*. Arteether, a potent semisynthetic analogue of dihydroartemisinin is being developed by the World Health Organization as the artemisinin derivative of choice for the treatment of malaria. All three agents in doses of 400 and 600 mg/kg body weight were found to exhibit marked suppression of humoral responses, as measured by the hemolytic plaque assay, with arteether being the most potent. These agents did not alter the delayed-type hypersensitivity response to sheep erythrocytes at the same dose levels. In addition, all three agents were found not to possess any anti-inflammatory activity when tested on carrageenan-induced oedema. These results indicated that these agents have a selective immunosuppressive activity. They did not exhibit immunostimulating activity in contrast to what has been reported for sodium artesunate.


Teoh K.H., Polichuk D.R., Reed D.W., Nowak G. & Covello P.S. (2006). “*Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin.” *FEBS Lett.* **580**(5), 1411-6. [Abstract]. Artemisinin, a sesquiterpene lactone endoperoxide derived from the plant *Artemisia annua*, forms the basis of the most important treatments of malaria in use today. In an effort to elucidate the biosynthesis of artemisinin, an expressed sequence tag approach to identifying the relevant biosynthetic genes was undertaken using isolated glandular trichomes as a source of mRNA. A cDNA clone encoding a cytochrome P450 designated CYP71AV1 was characterized by expression in *Saccharomyces cerevisiae* and shown to catalyze the oxidation of the proposed biosynthetic intermediates amorpha-4,11-diene, artemisinic alcohol and artemisinic aldehyde. The identification of the CYP71AV1 gene should allow for the engineering of semi-synthetic production of artemisinin in appropriate plant or microbial hosts.


alpha-Artelinic acid [8], a potent, stable, and water-soluble antimalarial agent, has been synthesized from artemisinin [1], the sesquiterpene lactone endoperoxide isolated from Artemisia annua. The blood schizontocidal antimalarial activity of alpha-artelinic acid evaluated against Plasmodium knowlesi is also reported.

Weathers P., Smith T., Hemmavanh D., Follansbee E., Ryan J. & Cheetham R. “The production of the antimalarial, artemisinin, by transformed roots of Artemisia annua.” ISHS Acta Horticulture 426: International Symposium on Medicinal Aromatic Plants. Abstract. Artemisinin is a potent antimalarial produced by transformed (hairy) roots of Artemisia annua. Studies were done to determine the effect of varying the strength of B5 culture media, source and level of nitrogen in the media (nitrate and ammonium), phosphate, and the phytohormone, gibberellic acid (GA3), on both biomass and artemisinin production in hairy root cultures. Preliminary results show that a 33% increase in B5 medium, an increase in nitrate, an elimination of ammonium, and addition of GA3 increase biomass yields. Artemisinin production was stimulated by regular strength B5 (with lower levels of nitrate than for maximum biomass), low levels of phosphate, and GA3.


Wright C.W. (2005) "antimalarials and the development of novel antimalarial drugs." J Ethnopharmacol. 22, 100(1-2), 67-71. Abstract. Malaria continues to be a major cause of mortality and morbidity especially throughout the developing world. In the last 25 years or so a number of significant advances have been made that have the potential to make a major contribution to the control of this disease. The discovery of artemisinin and its analogues as potent antimalarial agents have been of immense importance and the latter, as well as some other selected developments are outlined in this brief review.

Zhao K.C. & Song Z.Y. (1990) "The pharmacokinetics of dihydroqinghasu given orally to rabbits and dogs." Yao Xue Xue Bao 25(2), 147-9. Abstract. Qinghaosu (QHS), also known as artemisinine and arteannuin, is isolated from the Chinese herb Artemisia annua L. It is highly active against both chloroquine-sensitive and chloroquine-resistant strains of P. berghei and has been approved by the Ministry of Health for the treatment of malaria. When QHS is treated with sodium borohydride, dihydroqinghaosu (DH QHS) is resulted with the antimalarial activity enhanced several fold. This paper reports the pharmacokinetics of DHQHS studied with the radioimmunoassay method. When the drug was given orally in tablet form to rabbits at doses of 10, 20 and 30 mg/kg, peak serum levels of 0.03, 0.05 and 0.13 micrograms/ml, respectively, were obtained in 1 to 2 h. The corresponding T1/2 of the drug were found to be 1.19, 1.00 and 1.10 h and the MRTs were 1.73, 1.36 and 1.53 h. No significant difference between dosages used was observed. When dogs were given DHQHS tablets at the dose of 20
mg/kg, a peak serum concentration of 0.13 micrograms/ml was reached in about 2 h with a T1/2 of 2.10 h and an MRT of 3.04 h. However, when dogs were given QHS tablets at the dose of 70 mg/kg, no drug was detected in the serum. It would appear that the bioavailability of DHQHS tablets is much higher than that of QHS when given orally to the dog.

Other Anti-malarial Chinese Herbs.


Other Anti-Malarial Essential oil Studies.


Abstract. Since ancient times, plant products were used in various aspects. However, their use against pests decreased when chemical products became developed. Recently, concerns increased with respect to public health and environmental security requiring detection of natural products that may be used against insect pests. In this study, 41 plant extracts and 11 oil mixtures were evaluated against the yellow fever mosquito, *Aedes aegypti* (Linnaeus), the malaria vector, *Anopheles stephensi* (Liston), and the filariasis and encephalitis vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae) using the skin of human volunteers to find out the protection time and repellency. The five most effective oils were those of Litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*), which induced a protection time of 8 h at the maximum and a 100% repellency against all three species. This effect needs, however, a peculiar formulation to fix them on the human skin.


Abstract. In a search for new plant-derived biologically active compounds against malaria parasites, five essential oils extracted from the Cameroonian plants *Xylopia phloiodora*, *Pachypodanthium confine*, *Antidesma laciniatum*, *Xylopia aethiopica*, and *Hexalobus crispiflorus* were evaluated in regard to their anti-
plasmodial activity against the W2 strain of *Plasmodium falciparum*. The oils were obtained from the plants with 0.12, 0.13, 0.18, 0.6 and 0.1% yields (relatively to dried material weight) respectively. Analysis by gas chromatography and mass spectrometry identified mainly terpenoids, among which alpha-copaeone, gamma-cadinene, delta-cadinene, alpha-cadinol, spathulenol and caryophyllene oxide were most commonly found. The five oils were active against *Plasmodium falciparum* in culture. The most effective was the oil of *Hexalobus crispiflorus*, with an IC$_{50}$ of 2 microg/ml.

Burfield T. & Reekie S.-L. (2000) "Mosquitoes, malaria & essential oils." *International J. of Aromatherapy* **15**(1), 30-41. Abstract. Biocidal (insect repellent) preparations used against mosquito bites to prevent infection are reviewed, comparing the use of essential oils and natural aromatic materials with various synthetic topical agents. A synopsis of malaria prevention strategies and insecticidal toxicity is also discussed, in the light of emergent mosquito resistance to synthetic chemical pesticides. The use of natural products for use as anti-malarials is also reviewed, finding that a serious divide occurs between a purely open approach, and the approach via the commercial interests of pharmaceutical and chemical companies. The latter tend to focus on the exploitation of single active chemicals, whilst the activity of whole botanical extracts is overlooked despite the fact that insect resistance to single actives is common; and resistance to whole plant extracts is rare. The European Union’s role in this issue is also discussed. Better formulation technology is needed for topical repellents by providing more effective fixation for the essential oil content and incorporating strategies for controlled release of essential oil vapours, whilst providing solutions for the problem of potential dermal irritancy.

Campbell W.E., Gammon D.W., Smith P., Abrahams M. & Purves T.D. (1997). “Composition & anti-malarial activity in vitro of the essential oil of *Tetradenia riparia*.” *Planta Med.* **63**, 270-272. Abstract. The essential oil from the leaves and stems of *Tetradenia riparia* was analysed by GC and GC/MS and 35 components were identified. The main constituents were alpha-terpineol (22.6%), fenchone (13.6%), beta-fenchyl alcohol (10.7%), beta-caryophyllene (7.9%), and perillyl alcohol (6.0%). Moderate antimalarial activities were recorded against two strains of *Plasmodium falciparum*.

sit D, Choochote W, Rattanachanpichai E, Chaithong U, Chaiwong P, Jitpakdi A, Tippawangkosol P, Riyong D, Pitasawat B. (2006) "Essential oils as potential adulticides against two populations of *Aedes aegypti*, the laboratory and natural field strains, in Chiang Mai province, northern Thailand." *Parasitol Res.* **99**(6), 715-21. Abstract. Essential oils derived from five plant species, celery (*Apium graveolens*), caraway (*Carum carvi*), zedoary (*Curcuma zedoaria*), long pepper (*Piper longum*), and Chinese star anise (*Illicium verum*), were subjected to investigation of adulticidal activity against mosquito vectors. Two populations of *Aedes aegypti*, the laboratory and natural field strains, collected in Chiang Mai province, northern Thailand were tested in pyrethroid-susceptibility bioassays. The results revealed that the natural field strain of *A. aegypti* was resistant to
permethrin, with mortality rates ranging from 51 to 66%. A mild susceptibility, with mortality rates ranging from 82 to 88%, was observed in the natural field strain of A. aegypti exposed to lambdacyhalothrin, which suggested that this strain was tolerant and might be resistant to this insecticide. However, laboratory-reared A. aegypti exposed to discriminating dosages of permethrin and lambdacyhalothrin induced 100% mortality in all cases, thus indicating complete susceptibility of this strain to these insecticides. The adulticidal activity determined by topical application revealed that all five essential oils exerted a promising adulticidal efficacy against both laboratory and natural field strains of A. aegypti. Although the laboratory strain was slightly more susceptible to these essential oils than the natural field strain, no statistically significant difference was observed. Moreover, comparison of the adulticidal activity indicated that the performance of these essential oils against the two strains of A. aegypti was similar. The highest potential was established from caraway, followed by zedoary, celery, long pepper, and Chinese star anise, with an LC(50) in the laboratory strain of 5.44, 5.94, 5.96, 6.21, and 8.52 microg/mg female, respectively, and 5.54, 6.02, 6.14, 6.35, and 8.83 microg/mg female, respectively, in the field strain. These promising essential oils are, therefore, an alternative in developing and producing mosquito adulticides as an effective measure used in controlling and eradicating mosquito vectors.

Coppi A., Cabinian M, Mirelman D, Sinnis P. (2006) "Antimalarial activity of allicin, a biologically active compound from garlic cloves." Antimicrob Agents Chemother. 50(5), 1731-7. Abstract. The incidence of malaria is increasing, and there is an urgent need to identify new drug targets for both prophylaxis and chemotherapy. Potential new drug targets include Plasmodium proteases that play critical roles in the parasite life cycle. We have previously shown that the major surface protein of Plasmodium sporozoites, the circumsporozoite protein (CSP), is proteolytically processed by a parasite-derived cysteine protease, and this processing event is temporally associated with sporozoite invasion of host cells. E-64, a cysteine protease inhibitor, inhibits CSP processing and prevents invasion of host cells in vitro and in vivo. Here we tested allicin, a cysteine protease inhibitor found in garlic extracts, for its ability to inhibit malaria infection. At low concentrations, allicin was not toxic to either sporozoites or mammalian cells. At these concentrations, allicin inhibited CSP processing and prevented sporozoite invasion of host cells in vitro. In vivo, mice injected with allicin had decreased Plasmodium infections compared to controls. When sporozoites were treated with allicin before injection into mice, malaria infection was completely prevented. We also tested allicin on erythrocytic stages and found that a 4-day regimen of allicin administered either orally or intravenously significantly decreased parasitemias and increased the survival of infected mice by 10 days. Together, these experiments demonstrate that the same cysteine protease inhibitor can target two different life cycle stages in the vertebrate host.

Emeje M.O., Isimi C.Y., Oqua D.A., Kunle O.O. (2005) "Some compaction characteristics of the hot water leaf extract of Nauclea latifolia: a potential antimalarial agent." J. Herb. Pharmacother. 5(4), 23-30 Abstract. This study aims to develop a suitable tablet dosage form of Nauclea latifolia, a potential antimalarial agent. The compaction characteristics of the oven dried water extract were studied using the Heckel equation. The mechanical properties of the compacts were also determined. This preliminary information will be useful in developing a suitable dosage form of the extract for use in the management of malaria. The results showed that N. latifolia extract exhibited high densification due to dye filling while the subsequent rearrangement of the granules did not contribute, significantly, to their densification. The granules had enhanced plasticity as shown by the low yield point, Py. The tablets produced from the extract had good mechanical properties, with hardness increasing via compression pressure while the friability decreased. However, the tablets had poor disintegration properties; it is concluded that while tablets of suitable physical properties can be produced from the extract, a disintegrant would need to be included in the formulation to ensure adequate drug release.

Garavito G., Rincon J., Arteaga L., Hata Y., Bourdy G., Gimenez A., Pinzon R. & Deharo E. (2006), “Antimalarial activity of some Colombian medicinal plants.” J Ethnopharmacol. 11,107(3), 460-2. Abstract. Antimalarial activity of 10 vegetal extracts (9 ethanolic extracts and 1 crude alkaloid extract), obtained from eight species traditionally used in Colombia to treat malaria symptoms, was evaluated in culture using Plasmodium falciparum chloroquine resistant (FcB2) strain and in vivo on rodent malaria Plasmodium berghei. The activity on ferritropophorphyrin biomineralization inhibition test (FBIT) was also assessed. Against Plasmodium falciparum, eight extracts displayed good activity Abuta grandifolia (Mart.) Sandwith (Menispermaceae) leaves, Acacia farnesiana (L.) Willd. (Mimosaceae) leaves, Acnistus arborescens (L.) Schltdl. (Solanaceae) aerial part, Croton leptostachyus Kunth (Euphorbiaceae) aerial part, Piper cumanense Kunth (Piperaceae) fruits and leaves, Piper holtonii C. DC. (Piperaceae) aerial part and Xylopia aromatica (Lam.) Mart. (Annonaceae) bark with IC(50) values ranging from <1 to 2.1 mug/ml, while in the in vivo model only Abuta grandifolia alkaloid crude extract exhibits activity, inhibiting 66% of the parasite growth at 250 mg/kg/day. In the FBIT model, five extracts were active (Abuta grandifolia, Croton leptostachyus, Piper cumanense fruit and leaves and Xylopia aromatica).

Hout S., Chea A., Bun S.S., Elias R., Gasquet M., Timon-David P., Balansard G. & Azas N. (2006) “Screening of selected indigenous plants of Cambodia for antiplasmodial activity.” J. Ethnopharmacol. 11,107(1), 12-8. Abstract. The in vitro antiplasmodial activity of 117 aqueous, methanol and dichloromethane extracts derived from different parts of 28 indigenous wild plant species was studied. These plants are commonly used in Cambodian traditional medicine. The plant extracts were tested for in vitro activity against a chloroquine resistant Plasmodium falciparum strain (W2). Nine extracts were moderately active with IC(50) values ranging between 5 and 10 microg/ml, 17 extracts were active with
IC(50) values ranging between 1 and 5 microg/ml. These 26 extracts derived from eight plants belong to six families. The most active extracts were dichloromethane and came from *Stephania rotunda* and *Brueca javanica* with IC(50) values of 1 microg/ml and a selectivity index > or = 25. It is interesting to note that some aqueous extracts were as active as dichloromethane extracts especially aqueous extracts of *Stephania rotunda*, *Brueca javanica*, *Phyllanthus urinaria* and *Eurycoma longifolia* with IC(50) values of < or = 4 microg/ml. These results are in agreement with statements of healers on traditional uses of these plants for the treatment of malaria and/or fever. In this study, we report the antiplasmodial potential activity of eight plant species from Cambodia. Among them four are tested for the first time.


Jullian V., Bourdy G., Georges S., Maurel S. & Sauvain M. (2006) "Validation of use of a traditional antimalarial remedy from French Guiana, *Zanthoxylum rhoifolium* Lam." *J. Ethnopharmacol.* 19, 106(3), 348-52. Abstract. *Zanthoxylum rhoifolium* bark (Rutaceae) is a medicinal plant, traditionally used in French Guiana to treat and prevent malaria. Bioassay-guided extractions of *Zanthoxylum rhoifolium* bark have shown that antiplasmodial activity is concentrated in the alkaloid fraction. Further fractionation of this extract has yielded seven benzophenanthridine alkaloids, dihydroavicine 1, dihydronitidine 2, oxyavicne 3, oxynitidine 4, fagaridine 5, avicine 6 and nitidine 7. Antimalarial activity of the last five compounds has been evaluated, and nitidine was the most potent, displaying an IC(50)<0.27microM against *Plasmodium falciparum*. Investigation of the traditional remedy, a trunk bark decoction in water, has shown that fagaridine 5, avicine 6 and nitidine 7 are also present in the decoction, therefore justifying the traditional use of *Zanthoxylum rhoifolium* bark as antimalarial.

Kalauni S.K., Awale S., Tezuka Y., Banskota A.H., Linn T.Z., Asih P.B., Syafruddin D. & Kadota S. (2006) Antimalarial activity of cassane- and norcassane-type diterpenes from *Caesalpinia crista* and their structure-activity relationship. *Biol Pharm Bull.* 29(5), 1050-2. Abstract. Malaria is one of the most life-threatening infectious diseases worldwide and claims millions of people’s lives each year. The appearance of drug-resistance *Plasmodium falciparum* has made the treatment of malaria increasingly problematic, and thus, it is a dire need to search the new alternatives of current drugs. In the present study, 44 cassane- and norcassane-type diterpenes isolated from *Caesalpinia crista* of Myanmar and Indonesia were evaluated for their antimalarial activity against the malaria parasite *Plasmodium falciparum* FCR-3/A2 clone in vitro. Most of the tested diterpenes displayed antimalarial activity, and norcaesalpinin E (28) showed the most potent activity with an IC_{50} value of 0.090 microM, more potent than the clinically used drug chloroquine (IC_{50}, 0.29 microM). Based on the observed results, a structure-activity relationship has been established.
Kirira P.G., Rukunga G.M., Wanyonyi A.W., Muregi F.M., Gathirwa J.W., Muthaura C.N., Omar S.A., Tolo F., Mungai G.M. & Ndiege I.O. (2006) "Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya." *J. Ethnopharmacol.* 19, **106**(3), 403-7. **Abstract.** The methanol and aqueous extracts of 10 plant species (*Acacia nilotica*, *Azadirachta indica*, *Carissa edulis*, *Fagaropsis angolensis*, *Harrissonia abyssinica*, *Myrica salicifolia*, *Neoboutonia macrocalyx*, *Strychnos heningsii*, *Withania somnifera* and *Zanthoxylum usambarensis*) used to treat malaria in Meru and Kilifi Districts, Kenya, were tested for brine shrimp lethality and in vitro anti-plasmodial activity against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* (NF54 and ENT30). Of the plants tested, 40% of the methanol extracts were toxic to the brine shrimp (LD(50)<100micro/ml), while 50% showed in vitro anti-plasmodial activity (IC(50)<100microg/ml). The methanol extract of the stem bark of *N. macrocalyx* had the highest toxicity to brine shrimp nauplii (LD(50) 21.04+/-1.8microg/ml). Methanol extracts of the rest of the plants exhibited mild or no brine shrimp toxicity (LD(50)>50microg/ml). The aqueous extracts of *N. macrocalyx* had mild brine shrimp toxicity (LD(50) 41.69+/-0.9microg/ml), while the rest were lower (LD(50)>100microg/ml). The methanol extracts of *F. angolensis* and *Zanthoxylum usambarensis* had IC(50) values <6microg/ml while the aqueous ones had values between 6 and 15microg/ml, against both chloroquine-sensitive and resistant *P. falciparum* strains. The results support the use of traditional herbs for anti-malarial therapy and demonstrate their potential as sources of drugs.

Kraft C., Jenett-Siems K., Siems K., Jakupovic J., Mavi S., Bienzle U. & Eich E. (2003) "In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe." *Phytother Res.* 17(2), 123-8. **Abstract.** In this study the in vitro antiplasmodial activities of extracts from *Cussonia spicata* (Araliaceae), *Artemisia afra*, *Vernonia colorata*, *V. natalensis* (Asteraceae), *Parinari curatellifolia* (Chrysobalanaceae), *Clutia hirsuta*, *Flueggea virosa* (Euphorbiaceae), *Adenia gummiifera* (Passifloraceae) and *Hymenodictyon floribundum*, (Rubiaceae) were evaluated. The lipophilic extracts from the aerial parts of *Artemisia afra* and *Vernonia colorata* proved to be the most active against the chloroquine-sensitive strain PoW and against the chloroquine-resistant clone Dd2 of *Plasmodium falciparum*. Bioassay-guided fractionation of the extract of *A. afra* yielded seven flavonoids, from which acacetin, genkwanin and 7-methoxyacacetin showed in vitro activity; the IC(50) values ranged from 4.3 microgram/mL to 12.6 microgram/mL. In addition, several sesquiterpene lactones could be obtained from the most active fractions. Whereas eudesmaafragluacolide proved to be inactive, the guaianolides 1-desoxy-1alpha-peroxy-rupticolin A-8-O-acetate, 1-alpha,4-alpha-dihydroxybishopsolepoxide and rupicolin A-8-O-acetate revealed in vitro antiplasmodial activity. Evaluation of *V. colorata* gained four sesquiterpenes 11-beta,13-dihydrovermodalin, vernodalol, 11-beta,13-dihydrovermolide and 11-beta,13,17,18-tetrahydrovermolide, from which the first two constituents exhibited the strongest antiplasmodial activity (IC(50) values: 1.1-4.8 microgram/mL).
Kvist L.P., Christensen S.B., Rasmussen H.B., Mejia K. & Gonzalez A. (2006) "Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis" J. Ethnopharmacol. 19, 106(3), 390-402. Abstract. Households in eleven geographically and ethnically distinct areas in Loreto, Peru, were interviewed about their knowledge and use of plants, for the treatment of malaria and leishmaniasis. The survey resulted in 988 use records representing 118 plant-taxa for malaria and 289 use-records representing 85 plant-taxa for leishmaniasis. In both cases the 10 most frequently reported taxa accounted for about half of all the use-records. Plant material was collected and extracts were screened for in vitro inhibition of *Plasmodium* and *Leishmania* parasites. In the case of *Plasmodium*, extracts of 11 of the 13 most frequently reported plants showed significant growth inhibitory activity, while only a few plant extracts inhibited the growth of *Leishmania* parasites.

Lopes N.P., Kato M.J., de A. Andrade E.H., 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 Walapai Amazon Indians." J. Ethnopharmacol 67, 313-319. Abstract. The Amazon Indians Waia̱pi living in the West of Amapá State of Brazil, treat malaria with an inhalation of vapor obtained from leaves of *Viola surinamensis*. The essential oil obtained from adult and plantlet leaves was analyzed by GC:MS and 11 monoterpenes, 11 sesquiterpenes and three phenylpropanoids were identified. Plantlet essential oil caused 100% of growth inhibition after 48 h in the development of the young trophozoite to schizont stage and the sesquiterpene nerolidol (100 µg/ml) was identified as one of the active constituents (100% of growth inhibition was obtained). In addition, examination of [U14C]-glucose incorporation showed that activity of nerolidol is related to the inhibition of glycoprotein biosynthesis.

Lopes NP, Kato MJ, Andrade E.H de A., Maia JGS, Yoshida M., Planchart A.R. & Katzin A.M (1999) "Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiãpi Amazon Indians." Journal of Ethnopharmacology 67(3),313-319. Abstract. The Amazon Indians Waiãpi living in the West of Amapá State of Brazil, treat malaria with an inhalation of vapor obtained from leaves of *Viola surinamensis*. The essential oil obtained from adult and plantlet leaves was analyzed by GC/MS and 11 monoterpenes, 11 sesquiterpenes and three phenylpropanoids were identified. Plantlet essential oil caused 100% of growth inhibition after 48 h in the development of the young trophozoite to schizont stage and the sesquiterpene nerolidol (100 µg/ml) was identified as one of the active constituents (100% of growth inhibition was obtained). In addition, examination of [U14C]-glucose incorporation showed that activity of nerolidol is related to the inhibition of glycoprotein biosynthesis.


Sixty-six extracts of 18 plants commonly used by traditional healers in Congo Brazzaville for the treatment of malaria have been investigated for in vitro antiplasmodial activity. Ethanolic and dichloromethane extracts of 7 among the 18 studied plants were moderately active (10 microg/ml < IC(50) < 50 microg/ml). These extracts concerned Cassia siamea (bark), Cogniauxia podolaena (root), Landolphia lanceolata (root and leaves), Millettia versicolor (leaves), Pseudospondias microcarpa (leaves), Uapaca paludosa (leaves) and Vernonia brazzavillensis (leaves). These results support their traditional use as antimalarial plants. The bark extract of Uapaca paludosa showed a good activity (<10 microg/ml) and the extracts from Quassia africana (root and leaves) even exhibited IC(50) values less than 1 microg/ml. Except for Quassia africana, for which the three solvents (water, ethanol and dichloromethane) present an effective extraction, no aqueous extract was highly active. The cytotoxicity of aqueous, DCM and ethanol extracts of Quassia africana was tested on KB cell lines.

Menan H., Banzouzi J.T., Hocquette A., Pelissier Y., Blache Y., Kone M., Mallie M., Assi L.A. & Valentin A. (2006) "Antiplasmodial activity and cytotoxicity of plants used in West African traditional medicine for the treatment of malaria." J. Ethnopharmacol. 21, 105(1-2), 131-6. Abstract. Eighteen plants originating from Ivory Coast were selected by ethnobotanical survey as plants commonly used by traditional healers for the treatment of malaria. Extracts of these plants were tested on two strains of Plasmodium falciparum: FcM29-Cameroon (chloroquine-resistant strain) and a Nigerian chloroquine-sensitive strain. The powdered plants were used to prepare three kinds of extracts: by decoction in water, in ethanol (95%) and in pentane. A radioactive micromethod allowed the evaluation of the antiplasmodial in vitro activity of the extracts on P. falciparum. Concentrations inhibiting 50% of the parasite growth (IC50) ranged from 18 microg/ml to more than 500 microg/ml for aqueous and ethanol extracts and from 4.3 microg/ml to more than 500 microg/ml for pentane extracts. Cytotoxicity was estimated on A375 melanoma cells and a cytotoxicity/antiplasmodial index (CAR) was calculated for each extract, ranging from 1 to 10. The pentane extracts of Cola caricaefolia and Uvaria afzelii, which revealed the strongest antiplasmodial activity had CAR values of about 10.

de Mendonça F.A.C., da Silva K.F.S., dos Santos K.K., Ribeiro Jr K.A.L. & Sant'Ana A.E.G. (2005) "Activities of some Brazilian plants against larvae of the mosquito Aedes aegypti." Fitoterapia 76 (7-8), 629-636. Abstract. The insecticidal activities of extracts and oils of seventeen medicinal plants of Brazil have been determined using an Aedes aegypti larvicidal bioassay. Oils from Anacardium occidentalis, Copaifera langsdorffii, Carapa guianensis, Cymbopogon winterianus and Ageratum conyzoides showed high activities with LC50 values of 14.5, 41, 57, 98 and 148 µg/l, respectively. The most active ethanolic extract tested was that from the stem of Annona glabra which presented an LC50 value of 27 µg/l. The potential application of cashew nut oil, an industrial by-product with low commercial value, in the control of the vector of dengue and yellow fever, may be proposed.
Milhau G., Valentin A., Benoit F., Mallie M., Bastide J.M., Pelissier Y. & Bessiere J.M. (1997) "In vitro antimalarial activity of eight essential oils." *Journal of Essential Oil Research* **9**(3), 329-333. **Abstract.** Essential oils of eight plants were prepared by hydrodistillation of leaves and twigs (stems) of *Artemisia vulgaris*, *Eucalyptus globulus*, *Myrtus communis*, *Juniperus communis*, *Lavandula angustifolia*, *Origanum vulgare*, *Rosmarinus officinalis* and *Salvia officinalis*. Their oil components were characterized by GC and MS. These oils were then tested for in vitro antimalarial activity on *Plasmodium falciparum*. Two strains of *P. falciparum* were tested: FcB1-Columbia (chloroquine-resistant) and a Nigerian chloroquine-sensitive strain. Concentrations inhibiting 50% of the parasite in vitro growth, obtained after 24 and 72 h contact between the oil and the parasite culture, ranged from 150 microgram/mL to 1 mg/mL. The best results were obtained with *Myrtus communis* and *Rosmarinus officinalis* oils which inhibited *P. falciparum* at concentrations ranging from 150 to 270 microgram/mL.

Moore SJ, Hill N, Ruiz C, Cameron MM. (2007) "Field evaluation of traditionally used plant-based insect repellents and fumigants against the malaria vector *Anopheles darlingi* in Riberalta, Bolivian Amazon." *J Med Entomol.* **44**(4), 624-30. **Abstract.** Inexpensive insect repellents may be needed to supplement the use of impregnated bed-nets in the Amazon region, where the primary malaria vector, *Anopheles darlingi* (Root), is exophilic and feeds in the early evening. Three plants that are traditionally used to repel mosquitoes in Riberalta, Bolivian Amazon, were identified by focus group, and then they were tested against *An. darlingi* as well as *Mansonia indubitans* (Dyar & Shannon)/*Mansonia titillans* (Walker). *Cymbopogon citratus* (Staph), Guatemalan lemongrass, essential oil at 25% was used as a skin repellent, and it provided 74% protection for 2.5 h against predominantly *An. darlingi* and 95% protection for 2.5 h against *Mansonia* spp. *Attalea princeps* (name not verified) husks, burned on charcoal in the traditional way provided 35 and 51% protection against *An. darlingi* and *Mansonia* spp., respectively. Kerosene lamps, often used to light rural homes, were used as a heat source to volatilize 100% *Mentha arvensis* (Maliv ex. Bailey) essential oil, and they reduced biting by 41% inside traditional homes against *Mansonia* spp., although they were ineffective outdoors against *An. darlingi*. All three plant-based repellents provided significant protection compared with controls. Plant-based repellents, although less effective than synthetic alternatives, were shown by focus groups to be more culturally acceptable in this setting, in particular para-menthane-3, 8, 10, idol derived from lemon eucalyptus, *Corymbia citriodora* (Hook). Plant-based repellents have the potential to be produced locally and therefore sold more cheaply than synthetic commercial repellents. Importantly, their low cost may encourage user compliance among indigenous and marginalized populations.

Abstract. Twenty-four kinds of water extracts derived from 22 plants that are traditionally used for the treatment of malaria on Java Island, Indonesia, were screened for their antibabesial and antimalarial activities. Among the extracts, 8 extracts displayed strong antimalarial activity, with an inhibition range from 89.6 to 100%, and 15 showed strong antibabesial activity, with an inhibition range from 84.2 to 98.1%. The extracts of *Achillea millefolium*, *Baeckea frutescens*, *Brucea javanica*, *Curcuma xanthorrhiza*, *Strychnos lucida* and *Swietenia macrophylla* showed both strong antibabesial and antimalarial activities. The antimalarial activities paralleled the antibabesial activities, but the converse was not true.

Nathan S.S., Kalaivani K. & Murugan K. (2005) "Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae)." *Acta Trop.* 96(1), 47-55. Abstract. The effects of the neem (*Azadirachta indica* A. Juss) limonoids azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin on *Anopheles stephensi* Liston (Diptera: Culicidae) were investigated. In exploring advantages of pure neem limonoids, we studied the larvicidal, pupicidal, adulticidal and antiovipositional activity of neem limonoids. Azadirachtin, salannin and deacetylgedunin showed high bioactivity at all doses, while the rest of the neem limonoids were less active, and were only biologically active at high doses. Azadirachtin was the most potent in all experiments and produced almost 100% larval mortality at 1 ppm concentration. In general, first to third larval instars were more susceptible to the neem limonoids. Neem products may have benefits in mosquito control programs.

Njoroge G.N. & Bussmann R.W. (2006) "Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya)." *J Ethnobiol Ethnomedicine.* 1, 2-8. Abstract. Plants in Kenya are becoming increasingly important as sources of traditional medicines. The World Health Organization (WHO) has estimated that malaria kills about 2.7 million people every year, 90% of who are from Africa. Malaria continues to be a national concern in Kenya as it plays a major role in the high mortality rates being experienced currently. The use and miss-use of chloroquine to prevent and treat falciparum malaria has led to widespread appearance of chloroquine resistant parasites in Kenya and other tropical countries. These factors and the rising costs of non-chloroquine drugs have made the local people to turn to traditional remedies for management of this menace. This paper examines the current utilization of traditional plant medicines in managing malaria menace in Central Kenya. The results show both indigenous and introduced species are in use indicating traditional medicinal practices in this region are dynamic. In total 58 species in 54 genera and 33 families were identified. The family Rubiaceae was found to have the highest number of reported species. Use of the various taxa is compared between five districts within Central Province of Kenya. The commonest species in this pharmacopoeia are: *Caesalpinia volkensii* Harms, *Strychnos henningsii* Gilg, *Ajuga remot*a Benth., *Warbugia ugandensis* Sprague and *Olea europaea* L. The first three species are used in all the five districts.
while the others are restricted in some of the districts. In 74% of the anti-malarial plant species reported in this study, the remedies are obtained in destructive manner and may need conservation measures to ensure sustainable utilization. The results of this study become a basis for selecting plants for further pharmacological and phytochemical studies in developing new and locally relevant anti-malarial agents.

Okkon J.E., Ita B.N. & Udokpoh A.E. (2006). "The in-vivo antimalarial activities of Uvaria chamae and Hippocratea africana." Ann Trop Med Parasitol. 100(7), 585-90. Abstract. The antimalarial activities of ethanolic root extracts of two plants used traditionally as malarial remedies in southern Nigeria, Uvaria chamae (Annonaceae) and Hippocratea africana (Hippocrateaceae), were studied in vivo, in mice infected with Plasmodium berghei berghei. The extract of U. chamae, when given orally at 300-900 mg/kg.day, exhibited significant antimalarial activity against both early and established infections. When established infections were treated, the mean survival time of the mice observed with this extract at 900 mg/kg.day was similar to that seen with the positive control: chloroquine at 5 mg/kg.day. The extract of H. africana, tested at oral doses of 200-600 mg/kg.day, also demonstrated promising blood schizontocidal activity, both in early and established infections. Although the question of their toxicities has still to be fully addressed, it is clear that both U. chamae and H. africana possess considerable antimalarial activity and they, or drugs based on their antimalarial constituents, may prove useful in the treatment of human malaria.

Okpako L.C. & Ajaiyeoba E.O. (2004). "In vitro and in vivo antimalarial studies of Striga hermonthica and Tapinanthus sessilifolius extracts." Afr J Med Med Sci. 33(1), 73-5. Abstract. The antimalarial activities of the methanol extracts of Striga hermonthica (whole plant) and Tapinanthus sessilifolius (leaves), commonly used in Northern Nigeria for the treatment of malaria, were evaluated. In the in vitro antiplasmodial analysis, the extracts of T. sessilifolius and S. hermonthica utilized in the study, displayed mild to weak activities with IC50 values of 200.5 and 274.8 microg/ml respectively. This was investigated, using the multidrug resistant Plasmodium falciparum, K1 strain, in the parasite lactate dehydrogenase assay. The murine model in vivo antimalarial activity of the tested extracts, using chloroquine-sensitive Plasmodium berghei (ANKA P1), in the 4-day suppressive test, showed that both plants had intrinsic antimalarial properties that were dose-dependent. At a dose of 400mg/kg weight of mice, extract of S. hermonthica exhibited a higher intrinsic antimalarial activity (68.5 % suppression) than that of T. sessilifolius (51.3 %). Chloroquine, the standard reference drug, had an average suppression of 78.0 % at a dose of 10 mg/kg weight of mice while normal saline was used as control. Preliminary phytochemical screening of the extracts indicated the presence of saponins, tannins, flavonoids, volatile oils and cardiac glycosides.

The plant terpenoids encompass a diversity of structures and have many functional roles in nature, including protection against pest arthropods. Previous studies in this laboratory have identified naturally occurring sesquiterpenes contained in essential oils from two plants, amyris (Amyris balsamifera) and Siam-wood (Fokienia hodginsii), that are significantly repellent to a spectrum of arthropod pests. In efforts to further examine the biological activity of this class of compounds 12 of these plant-derived sesquiterpenes have been isolated, purified, and assayed for spatial and contact repellency against the yellow fever mosquito, Aedes aegypti. These data were used to develop quantitative structure−activity relationships that identified key properties of the sesquiterpene molecule, including electronic and structural parameters that were used to predict optimal repellent activity. There were notable similarities in the models developed for spatial repellency over five time points and for contact repellency. Vapor pressure was an important component of all repellency models. Initial levels of spatial repellency were also related to polarizability of the molecule and lowest unoccupied molecular orbital (LUMO) energy, whereas the equation for late spatial repellency was dependent on other electronic features, including Mulliken population and electrotopological state descriptors. The model identified for contact repellency was the best fit and most significant model in this analysis and showed a relationship with vapor pressure, Mulliken population, and total energy.

Paluch G. & Coats J. (2007) "Mosquito and tick repellency to plant essential oils." Entomological Soc Annual Meeting Dec 10th 2007 Abstract. Screening trials in the Pesticide Toxicology Laboratory at Iowa State University have focused on characterizing the repellent activity of plant essential oils including catnip (Nepeta cataria), Osage orange (Maclura pomifera), West Indian sandalwood (Amyris balsamifera), and Siam wood (Fokienia hodginsii) against ticks and mosquitoes, including Culex and Aedes sp. Several major components of these essential oils were also isolated and evaluated for repellent activity. Tick climbing assays were designed to assess repellency by measuring responses to a barrier treated with an essential oil. Mosquito repellency was evaluated using a standardized static-air olfactometer. These studies and others have led to the development of mixtures containing different spatial and contact repellent activities. Mixtures consisting of essential oils and some isolated components from catnip, Osage orange, West Indian sandalwood and Siam wood oils, were found to have high residual repellent activity. All mixtures containing both contact and spatial mosquito repellents showed significantly higher levels of repellency as compared to the individual components or oils. Discussion will include trends observed in repellency screening trials regarding the potency of the parent essential oils to their major components and optimization of repellent blends, including residual control.

Lippia (Verbenaceae) as used in the South and Central America, and Tropical Africa, were revised and compared. A survey of the available literature shows that these species are used mostly for the treatment of gastrointestinal and respiratory disorders and as seasoning. Additionally, some of these Lippia species showed antimalarial, spasmolitic, sedative, hypotensive and, anti-inflammatory activities. Generally, the essential oil or the phenolic compounds (flavonoids) from these plant extracts are assumed to be the active principles.

Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi D, Rattanachenkichai E, Tippawangkosol P, Riyong D, Tuetsun B, Chaiyasit D. (2007) "Aromatic plant-derived essential oil: an alternative larvicide for mosquito control." *Fitoterapia.* 78(3), 205-10. Abstract: Five aromatic plants, *Carum carvi* (caraway), *Apium graveolens* (celery), *Foeniculum vulgare* (fennel), *Zanthoxylum limonella* (mullilam) and *Curcuma zedoaria* (zedoary) were selected for investigating larvicidal potential against mosquito vectors. Two laboratory-reared mosquito species, *Anopheles dirus*, the major malaria vector in Thailand, and *Aedes aegypti*, the main vector of dengue and dengue hemorrhagic fever in urban areas, were used. All of the volatile oils exerted significant larvicidal activity against the two mosquito species after 24-h exposure. Essential oil from mullilam was the most effective against the larvae of *A. aegypti*, while *A. dirus* larvae showed the highest susceptibility to zedoary oil.

Rodrigues Goulart H., Kimura E.A., Peres V.J., Couto A.S., Aquino Duarte F.A. & Katzin A.M. (2004) "Terpenes arrest parasite development & inhibit biosynthesis of isoprenoids in *Plasmodium falciparum.*" *AntiMicrob Agents & Chemotherapy.* 48(7), 2502-2509. Abstract: Development of new drugs is one of the strategies for malaria control. The biosynthesis of several isoprenoids in *Plasmodium falciparum* was recently described. Interestingly, some intermediates and final products biosynthesized by this pathway in mammals differ from those biosynthesized in *P. falciparum*. These facts prompted us to evaluate various terpenes, molecules with a similar chemical structure to the intermediates of the isoprenoids pathway, as potential antimalarial drugs. Different terpenes and S-farnesythiosalicylic acid were tested on cultures of the intraerythrocytic stages of *P. falciparum*, and the 50% inhibitory concentrations for each one were found: farnesol, 64 _M; nerolidol, 760 nM; limonene, 1.22 mM; linalool, 0.28 mM; and S-farnesythiosalicylic acid, 14 _M. All the terpenes tested inhibited dolichol biosynthesis in the trophozoite and schizont stages when [1-(n)-3H]farnesyl pyrophosphate triammonium salt ([3H]FPP) was used as precursor. Farnesol, nerolidol, and linalool showed stronger inhibitory activity on the biosynthesis of the isoprenic side chain of the benzoquinone ring of ubiquinones in the schizont stage. Treatment of schizont stages with S-farnesythiosalicylic acid led to a decrease in intensity of the band corresponding a p21ras protein. The inhibitory effect of terpenes and S-farnesythiosalicylic acid on the biosynthesis of both dolichol and the isoprenic side chain of ubiquinones and the isoprenylation of proteins in the intraerythrocytic stages of *P. falciparum* appears to be specific, because overall protein biosynthesis was not affected. Combinations of some terpenes or S-farnesythiosalicylic acid tested in this work
with other antimalarial drugs, like fosmidomycin, could be a new strategy for the treatment of malaria.

Schuster B.G. (2001) "Demonstrating the validity of natural products as anti-infective drugs." *J Altern Complement Med.* 2001; 7 Suppl 1, S73-82. **Abstract.** This presentation reviews the synthetic or classical development pathway of drug development and contrasts it with developing natural products as drugs. Also presented is an example of a traditional medicine that has been developed from a natural product and has become a "new/old" antiparasitic drug used in the treatment of malaria. The classic paradigm of synthetic drug development breaks down into drug discovery, drug design, preclinical studies, and clinical studies. This paradigm, constructed to weed out failures, results in a drug-development process that is high risk, time consuming, and expensive. The process requires screening an average of 10,000 active compounds to find a single compound that successfully makes its way through validation to drug approval and the marketplace. Following this paradigm, researchers progress from identifying a chemical lead to testing the compound in humans. The World Health Organization (WHO) Guidelines for the Assessment of Herbal Medicines are based on the classical guidelines and follow the classical approach to validating quality, safety, and efficacy--with one major difference. The starting point is to look at the natural product in humans. By taking into account the traditional experience with the product, the validation standard for safety and efficacy of natural products allows for the prolonged and apparently uneventful use of a substance to offer testimony of its safety. The reliance, then, is on experience--or what Western regulatory agencies would call "anecdotal information." Since most phytomedicines are a combination of several active ingredients, the WHO guidelines cover two kinds of combination products: Combinations that are already used in traditional medicine are considered "old" combination products. "New" combination products are well-known substances that are now being used in combination. *Artemisia annua*, a pervasive weed, has been referred to in Chinese medicine for thousands of years as a treatment for fever. In 1971, an extraction of artemisia yielded activity against *Plasmodium berghei*, a mouse model for malaria. The isolated compound, artemisinin, is an example of a traditional medicine that started out in humans, but which then provided a lead structure for a standard drug-development paradigm. Today, artemisinin derivatives are being used widely in combination therapy, especially in areas of the world where there is multidrug-resistant malaria.

Senthil Nathan S. (2007) "The use of *Eucalyptus tereticornis* Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae)." *Bioresour Technol.* 98(9), 1856-60. **Abstract.** Secondary metabolites obtained from the indigenous plants with proven mosquito control potential can be used as an alternative to synthetic insecticides under the integrated vector control. The essential oil extract from the forest redgum, *Eucalyptus tereticornis* Sm. (Myrtaceae) was tested against mature and immature mosquito vector *Anopheles stephensi* Liston (Diptera) under laboratory condition. The extract showed strong larvicidal, pupicidal and adulticidal activity.
The leaf oil extracts showed high bioactivity at high doses. Results obtained from the laboratory experiment showed that the leaf extracts suppressed the pupal and adult activity of *Anopheles stephensi* at higher doses. In general, first and second instar larvae were more susceptible to all treatments. Clear dose-response relationships were established with the highest dose of 160ppm plant extract evoking almost 100% mortality. The results obtained suggest that, in addition to their medicinal activities, *E. tereticornis* can also serve as a natural mosquitoicide.


Tagboto S. & Townson S. (2001) "Antiparasitic properties of medicinal plants and other naturally occurring products." *Adv Parasitol.* **50**, 199-295. **Abstract.** Parasitic diseases remain a major public health problem affecting hundreds of millions of people, particularly in tropical developing countries. The limited availability and affordability of pharmaceutical medicines means that the majority of the world's population depends on traditional medical remedies, and it is estimated that some 20,000 species of higher plant are used medicinally throughout the world. Many well-known drugs listed in the modern pharmacopoeia have their origins in nature, including, for example, quinine from the bark of the Cinchona tree for the treatment of malaria, which has been followed by the subsequent development of the synthetic derivatives chloroquine, amodiaquine, primaquine and mefloquine. More recently, the wider recognition of the antimalarial activity of artemisinin from the herb *Artemisia annua* has led current research to focus on the development of a large number of synthetic and semisynthetic compounds, which are more active than artemisinin. There is an increasing awareness of the potential of natural products, which may lead to the development of much-needed new antiparasitic drugs. In this chapter, we have drawn together a comprehensive list of medicinal plants and other natural products that have been shown to have activity against human and, to a lesser extent, animal parasites. In addition, some of the opportunities and difficulties in working with natural products have been reviewed and discussed, including the problems involved with evaluating complex mixtures of compounds which may occur in extracts, problems associated with differentiating between general cytotoxicity and genuine antiparasitic activity, and the hope that new technologies will rapidly accelerate new drug discovery and development in this field. Nevertheless, the way forward for natural product medicines, including the conservation of recognized natural products and protection of general biodiversity, the discovery and development process, and the promotion and usage of existing remedies, presents some difficult challenges. Following an initiative by the World Health Organization in August 2000, there is now the opportunity to evaluate scientifically many more traditional medicines and other natural products in validated antiparasite and toxicity screens, which will help establish which substances have potential for new pharmaceutical products. The use of 'untested' traditional medicines will no doubt continue, and there is an urgent need to distinguish between the efficacious and safe products and the
ineffective and/or unsafe products, particularly since many remedies are being more widely promoted in developing countries.

Taylor S. & Berridge V. (2006) "Medicinal plants and Malaria: an historical case study of research at the London School of Hygiene and Tropical Medicine in the twentieth century." Trans R Soc Trop Med Hyg. 100(8), 707-14. Abstract. Interest in medicinal plants has increased in recent years. This article examines the history of medicinal plant research through a case study of the London School of Hygiene and Tropical Medicine (LSHTM) over the past 100 years. Papers published by members of the School and documents in the School archives show a fluctuating but continuous interest in plants as sources of medicine. Research interests of individual scientists, changes in the School structure and the changing role of research affected research into medicinal plants at LSHTM. As important were external developments, including the supply of plant resources, especially during wartime, the development of drug-resistance, advances in science and technology, knowledge exchange between both disciplines and cultures, the increased influence of global organizations on policy, as well as pressure groups particularly those involved in conservation. With the revival of interest in plants and the increasing variety of influences on research, it is important to have a better understanding of how debates and subsequent policy impact at the research level, and how research in turn impacts upon policy.

Tchoumbougnang F., Zollo P.H., Dagne E. & Mekonnen Y. (2005) "In vivo antimalarial activity of essential oils from Cymbopogon citratus and Ocimum gratissimum on mice infected with Plasmodium berghei." Planta Med. 71(1), 20-23. Abstract. The essential oils obtained by hydrodistillation from fresh leaves of Cymbopogon citratus and Ocimum gratissimum growing in Cameroon were analyzed by GC and GC/MS. The main constituents of the oil of Ocimum gratissimum were gamma-terpinene (21.9 %), beta-phellandrene (21.1 %), limonene (11.4 %) and thymol (11.2 %), while the oil of Cymbopogon citratus contained geranial (32.8 %), neral (29.0 %), myrcene (16.2 %) and beta-pinene (10.5 %). The effects of these oils on the growth of Plasmodium berghei were investigated. Both oils showed significant antimalarial activities in the four-day suppressive in vivo test in mice. At concentrations of 200, 300 and 500 mg/kg of mouse per day, the essential oil of C. citratus produced the highest activity with the respective percentages of suppression of parasitaemia: 62.1 %, 81.7 % and 86.6 %. The corresponding values for the oil of O. gratissimum at the same concentrations were 55.0 %, 75.2 % and 77.8 %, respectively. Chloroquine (10 mg/kg of mouse, positive control) had a suppressive activity of 100 %.

seems to be an emerging tool to combat this vector. Piperitenone oxide isolated from essential oil of a new genotype, *Mentha spicata* L. variety *viridis*, has been evaluated for larvicidal, ovicidal, oviposition-deterrent, developmental toxicity, and repellent properties against various stages of *A. stephensi*. The results indicated the higher efficacy of piperitenone oxide than the crude essential oil of *M. spicata* variety *viridis* in all the bioassay experiments. The lethal response of piperitenone oxide and the oil toward fourth instar larvae showed LD$_{50}$ values of 61.64 and 82.95 microg/ml, respectively. Female adults of *A. stephensi* exposed to the oil laid approximately 42 times less number of eggs at the dose of 60.0 microg/ml as compared with control, whereas exposure of piperitenone oxide at the same dose completely inhibited the oviposition. Furthermore, piperitenone oxide also completely inhibited egg hatching at the dose of 75.0 microg/ml in ovicidal assay. Developmental toxicity studies showed the significant developmental inhibition potential of the compound and oil. Additionally, piperitenone oxide was found to be highly toxic and repellent toward adults of *A. stephensi* as compared with oil.

Trongtokit Y., Rongsriyam Y., Komalamisra N., Krisadaphong P. & Apiwathnasorn C. (2005) "Laboratory and field trial of developing medicinal local Thai plant products against four species of mosquito vectors." *Southeast Asian J Trop Med Public Health*. 35(2), 325-33. Abstract. Oils of *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* (makaen), widely used essential oils for dental caries or flavoring of food in Thailand, were prepared as 10 experimental repellent products in gel or cream form against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus* under laboratory conditions, using the human-arm-in-cage method. Two products that gave the longest-lasting complete protection were selected to examine their repellency against a variety of mosquito species under field conditions. In laboratory tests, 0.1 g of each product was applied to 3x10 cm of exposed area on a volunteer's forearm, while in field trials, 1.0 g was applied to each volunteer's leg (from knee to ankle). In the laboratory, the gel dosage form contained 20% clove oil (Gel B) or 10% clove plus 10% makaen oil mixture (Gel E) were promising plant-based repellents against three mosquito species and gave significantly longer complete protection times of 4-5 hours than all other developing products. Therefore, their efficacy in the field was evaluated. Under field conditions, Gel E showed complete protection for 4 hours and gave 95.7% repellency after 5 hours application, whereas Gel B and 20% deet (di-methyl benzamide) provided only 86.8 and 82.7% repellency after treatment, respectively against *Ae. aegypti*, daytime-biting mosquitos. For nighttime-biting, the 3 repellents under development yielded equally excellent (average 97.1%) repellency for 5 hours against the predominant *Cx. quinquefasciatus* and *Mansonia uniformis*, but they gave 89.0% repellency against *Cx. tritaeniorhynchus* and *Cx. gelidus*. This finding demonstrated the effectiveness of Gel B and Gel E products for possible use by low-income rural communities against various mosquito species.

Udeinya I.J., Brown N., Shu E.N., Udeinya F.I. & Quakeyie I. (2006) "Fractions of an antimalarial neem-leaf extract have activities superior to chloroquine, and are
gametocytocidal." *Ann Trop Med Parasitol.* **100**(1), 17-22. **Abstract.** The antimalarial activities of two fractions (IRDN-A and IRDN-B) of an extract from the leaves of the neem tree (*Azadirachta indica*) were compared with those of chloroquine, in in-vitro assays against *Plasmodium falciparum*. The asexual stages of a chloroquine-sensitive clone (ITG2F6) and a chloroquine-resistant isolate (W2) and the gametocytes of the NF 54 (BD-7) isolate of *P. falciparum* were used as the drug targets. Activity against the asexual stages was generally evaluated as the concentrations inhibiting the parasitaemias recorded in the control cultures, after an incubation of 48-72 h, by 50% (IC50) or 100% (IC100). For the ITG2F6 strain, the IC50 and IC100 (in microg/ml) were, respectively, 10(-5) and 10(-4) for IRDN-A, 10(-3) and 10(-2) for IRDN-B, and 10(-2) and 1.0 for chloroquine. The corresponding values for the W2 strain were 10(-5) and 1.0 for IRDN-A, and 10.0 and >100 for chloroquine (even at 100 microg/ml, chloroquine only inhibited the parasitaemia by 85%). Each of the two neem-leaf fractions lysed 50% and 100% of developing gametocytes, at 10(-3) and 1.0 microg/ml, respectively; and 50% and 100% of mature gametocytes at 10(-3) and 10(2) microg/ml, respectively. If they are found safe and effective in vivo, the neem-leaf fractions may form the basis of new antimalarial drugs that not only cure chloroquine-sensitive and chloroquine-resistant malaria but also markedly reduce transmission.

Valentin A., Pelissier Y., Benoit F., Marion C., Kone D., Mallie M., Bastide J.-M. & Bessière J.-M. (1995) "Composition & anti-malarial activity in vitro of volatile components of *Lippia multiflora*" *Phytochemistry* **40**, 1439-1442. **Abstract.** The essential oil of *Lippia multiflora* was prepared by hydrodistillation of the leaves & stalks and characterized by GC & mass-spectroscopy. The oil was tested for anti-malarial activity on in vitro cultures of *Plasmodium falciparum* (Fcb1-Columbia chloroquin-resistant strain and F32 - Tanzania chloroquin-sensitive strain). The dilutions inhibiting the in vitro growth of the parasite 50% 24 & 72hr after administration of the essential oil to the parasite culture were 1/12,000 and 1/21,000 respectively. When tested on highly synchronized culture, the essential oil inhibited growth mostly at the trophozoite-schizont step, indicating a potential effect on the first nuclear division of the parasite.

Vreden S.G., van den Broek M.F., Oettinger M.C., Verhave J.P., Meuwissen J,H, Sauerwein RW.(1992) "Cytokines inhibit the development of liver schizonts of the malaria parasite *Plasmodium berghei* in vivo." *Eur J Immunol.* **22**(9), 2271-5. **Abstract.** The effect of induction of an acute-phase response and its mediators on the development of liver schizonts of the rodent malaria parasite *Plasmodium berghei* was investigated in Brown Norway rats. Subcutaneous injection of turpentine oil 24 h or 5 min before inoculation of sporozoites resulted in 80% and 35% reduction of schizont development, respectively. Turpentine oil induced high plasma levels of interleukin-6 (IL-6). Intraperitoneal administration of IL-1, IL-6 or both, significantly reduced liver schizont development. This reduction was also present if IL-6 had been administered 24 h after sporozoite inoculation. Inhibition induced by IL-1 could be prevented by simultaneous administration of polyclonal anti-IL-6. Administration of polyclonal anti-IL-6 without IL-1 resulted in a 40%
increase of liver schizonts compared to control animals. We conclude that induction of an acute-phase response during experimental \textit{Plasmodium berghei} infections in Brown Norway rats, strongly inhibits liver schizont development and that IL-6 is a key mediator in this process.

Waako P.J., Smith P., & Folb P.I. (2005) "In vitro interactions of \textit{Aspilia africana} (Pers.) C.D. Adams, a traditional antimalarial medicinal plant, with artemisinin against \textit{Plasmodium falciparum}." \textit{J Ethnopharmacol.} 14, 102(2), 262-8. \textbf{Abstract.} Traditional antimalarial medicinal preparations are widely used \textit{africana} (Pers.) C.D. Adams is commonly used for traditional treatment of malaria symptoms in East and Central Africa. An in vitro study of interactions between an extract from this plant with artemisinin against two strains of \textit{Plasmodium falciparum} showed an antagonist relationship against both the chloroquine-sensitive D10 and the chloroquine- and sulphonamide-resistant K1 strains of \textit{Plasmodium falciparum}. The extract reduced accumulation of radiolabelled dihydroartemisinin ((3)H-DHA) by erythrocytes infected with the chloroquine- and sulphonamide-resistant K1 strain of \textit{Plasmodium falciparum} while it increased its accumulation by erythrocytes infected with the chloroquine-sensitive D10 strain. These results suggest complex interactions between the antimalarial medicinal plant and artemisinin. This study also proposes an in vitro approach to investigating interactions between antimalarial drugs and traditional medicines.


van Zyl R.L., Seatlholo S.T., van Vuuren S.F. & Viljoen A.M. (2006) “The biological activites of 20 nature identical essential oil constituents.” \textit{J. Essen. Oil Res.} 18, 129-133 (Special Edn. 2006). \textbf{Abstract:} Twenty nature identical essential oil constituents from seven structural groups were tested for anti-malarial, anti-microbial, antioxidant and toxicity properties. The compounds displayed anti-malarial activity (IC$_{50}$ values ranging from 0.9 to 1528.8µM) with varying toxicity (IC$_{50}$ values ranging from 5.5 to 1358.8µM). The data indicates that (-)-pulegone, (E- & Z-)-(±)-nerolidol, linalyl acetate & (+)-alpha-pinene have the most potent anti-malarial activity. Carvacrol (MIC values ranging from <1.66 to 13.3mM) followed by geraniol (MIC values 19.9-51.9mM) displayed the broadest spectrum of anti-microbial activity. The least toxic of the tested compounds, eugenol, was the only compound with antioxidant activity comparable to ascorbic acid. The results demonstrate that the 20 compounds individually display variable biological activity, although further research is required to determine how they may interact when combined.

Yadav S., Mittal P.K., Saxena P.N. & Singh R.K. (2008) "Effect of synergist piperonyl butoxide (PBO) on the toxicity of some essential oils against mosquito larvae." \textit{J Commun Dis.} 40(4), 263-8. \textbf{Abstract.} Effect of a known synergist piperonyl butoxide on the toxicity of steam distillate essential oils of Jamarosa (\textit{Cymbopogon nardus}), Pacholli (\textit{Pogostemon pacholl}), Basil (\textit{Ocimum basilicum}), and Peppermint (\textit{Mentha pipreta}) plant species against Anopheles stephensi larvae were evaluated. The purpose of the present study was to
identify the insecticidal potential of these oils against mosquito larvae. The Piperonyl Butoxide (PBO) was used to enhance the activity of these oils with the aim of developing essential oil based formulations. The bioassays of these oils with and without PBO were performed against late 3rd instar larvae of An. stephensi. The LC50 values against An. stephensi were 44.19 ppm for *Ocimum basilicum* oil, followed by, *Mentha piperita*, *Cymbopogon nardus*, and *Pogostemon pacholli* oil which gave LC50 values above 250 ppm. Thus in the present study the *Ocimum basilicum* oil was found to be most effective, whereas *Pogostemon pacholli* oil was found to least effective against mosquitoes for larvicidal action. The effect of synergist PBO led to the enhancement of toxicity of oils, the LC50 value for *Ocimum basilicum* were reduced from 44.19 ppm to 23.87 ppm. Similarly the oil of *Pogostemon pacholli* showed most significant results where the LC50 value was >250 ppm it was reduced to 50 ppm with PBO.

Zhu J., Zeng X., O’Neal M., Schultz G., Tucker B., Coats J., Bartholomay L.. & Xue R.-D. (2008) “Mosquito larvicidal activity of botanical-based mosquito repellents.”. *J. American Mosquito Control Association*. 24(1),161-68. Abstract. The larvicidal activity of 4 plant essential oils—cinnamon oil, lemon eucalyptus oil, sandalwood oil, and turmeric oil—previously reported as insect repellents was evaluated in the laboratory against 4th instars of *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens*. Sandalwood oil appeared to be the most effective of the larvicides, killing larvae of all 3 mosquito species in relatively short times. The values of LT50 and LT90 at the application dosage (0.2 mg/ml) were 1.06 ± 0.11 and 3.24 ± 0.14 h for *Ae. aegypti*, 1.82 ± 0.06 and 3.33 ± 0.48 h for *Ae. albopictus*, and 1.55 ± 0.07 and 3.91 ± 0.44 h for Cx. pipiens, respectively. Chemical compositions of these essential oils were also studied, and the larvicidal activity of their major ingredient compounds was compared with that of each of the essential oils. The acute toxicity of the 4 essential oils to fathead minnows was also evaluated. The safe use of these natural plant essential oils in future applications of mosquito control was discussed. *Cropwatch Comments* The analysis details given for Sandalwood oil (allegedly *Santalum album*) suggest that this oil is not derived from a *Santalum* spp., but instead derives from West Indian Sandalwood oil (*Amyris balsamifera*). Thanks to Cheryll Williams for pointing this out to Cropwatch.

**Malaria & DDT.**
Asawasinopon R., Prapamontol T., Prakobvitayakit O., Vaneesorn Y., Mangklabruks A. & Hock B. (2006) "Plasma levels of DDT and their association with reproductive hormones in adult men from northern Thailand." *Sci Total Environ.* 15, 355(1-3), 98-105. Abstract. Historically, dichlorodiphenyltrichloroethane (DDT) was used in northern Thailand for malaria control and farming purposes. Several studies have investigated its effects on end points of adverse reproductive health outcomes. However, the few previous studies investigated hormonal effects in men and available data are inconclusive. The authors aimed to explore the main hypothesis that plasma DDT levels in adult men were associated with reproductive hormone levels. A cross-sectional study was performed of 97 adult men living in a highland village named Mae Sa
Mai, 35 km north of Chiang Mai, Thailand. Venous blood samples were collected for measuring plasma levels of DDT and its metabolites and reproductive hormones, including 17-beta-estradiol (E2), testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH). 1,1-Dichloro-2,2-di(4-chlorophenyl)ethylene (p,p'-DDE) and 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (p,p'-DDT) were detected in all plasma samples. p,p'-DDE had the highest level with a median of 4057.7 ng/g lipids and a relatively higher level compared with most other studies. Plasma p,p'-DDT levels were positively associated with years of residence (beta+SE=0.472+0.208, P=0.028) and years of DDT usage for farming (beta+SE=0.177+0.084, P=0.04). The remarkable findings were the negative association of plasma E2 levels with plasma p,p'-DDE levels (beta+SE=-7.093+2.899, P=0.016) and the positive association with plasma 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene (o,p'-DDE) levels (beta+SE=16.381+5.596, P=0.008) after adjusting for age and body mass index (BMI). However, these associations were rather weak. Our results suggest that these associations may reflect their different mechanisms of hormonal activities and they would be warrant further detail investigations.

Beard J; Australian Rural Health Research Collaboration. (2006) "DDT and human health" Sci Total Environ. 15, 355(1-3), 78-89. Abstract. 1,1,1-Trichloro-2,2'bis(p-chlorophenyl) ethane (DDT) was the first widely used synthetic pesticide and is extremely persistent in both the environment and the human body. The introduction of DDT revolutionised agricultural production and has been credited with the elimination of malaria from the United States and Europe. However, DDT is also known to have had major environmental consequences and has been associated with dramatic declines in many animal populations. Although DDT use has generally been restricted since the early 1970s, exposure to the pesticide remains widespread. In developed countries, slow elimination from the body means a large proportion of the population still have detectable levels of DDT, or its metabolite DDE, in their serum or adipose tissue. In developing countries, the pesticide continues to be used for vector control and a significant proportion of breast-fed babies has daily intakes above recommended levels. This review considers the epidemiological evidence for possible adverse effects of human exposure to DDT. Much of this research is weakened by methodological flaws. However, recent methods in breast cancer research using nested studies in cohorts with stored biological samples have allowed a more rigorous assessment of a putative role for DDT in disease aetiology. While DDT does not appear to play a causative role in breast cancer development, there is suggestive evidence for a role in the aetiology of other conditions such as pancreatic cancer, neuropsychological dysfunction, and reproductive outcomes. Research into these and other conditions would benefit from the same rigorous approaches used in breast cancer research. Until further high quality evidence is available, it is still too early, even 60 years after the introduction of this once ubiquitous chemical, to pass judgement on the role of DDT in a number of common diseases.
Bouwman H., Sereda B. & Meinhardt H.M. (2006) "Simultaneous presence of DDT and pyrethroid residues in human breast milk from a malaria endemic area in South Africa." *Environ Pollut.* **144**(3), 902-17. **Abstract.** DDT and pyrethroids were determined in 152 breast-milk samples from three towns in KwaZulu-Natal, South Africa, one of which had no need for DDT for malaria control. All compounds were found present in breast milk. Primiparae from one town had the highest mean SigmaDDT whole milk levels (238.23µg/l), and multiparae from the same town had the highest means for permethrin (14.51µg/l), cyfluthrin (41.74µg/l), cypermethrin (4.24µg/l), deltamethrin (8.39µg/l), and Sigmapyrethroid (31.5µg/l), most likely derived from agriculture. The ADI for DDT was only exceeded by infants from one town, but the ADI for pyrethroids was not exceeded. Since the ADI for DDT was recently reduced from 20 to 10µg/kg/bw, we suggest that this aspect be treated with concern. We therefore raise a concern based on toxicant interactions, due to the presence of four different pyrethroids and DDT. Breastfeeding however, remains safe under prevailing conditions.

Casimiro S., Coleman M., Mohloai P., Hemingway J. & Sharp B. (2006) "Insecticide resistance in *Anopheles funestus* (Diptera: Culicidae) from Mozambique." *J. Med Entomol.* **43**(2), 267-75. **Abstract.** Malaria control in southern Mozambique is currently by indoor residual carbamate insecticide treatment, with pyrethroid-treated bed-nets distributed to pregnant women and children under five in northern Mozambique. The susceptibility of *Anopheles funestus* s.s. to pyrethroid, carbamate, organochlorine, and organophosphorus insecticides was determined by World Health Organization adult mosquito susceptibility tests at 19 localities in Mozambique, from March 2000 to July 2002. Biochemical assays were carried out on mosquitoes from the same families to detect shifts in the quantity or activity of enzyme families involved in insecticide detoxification. *An. funestus* from all localities remained fully susceptible to DDT and the organophosphorus insecticide malathion. A high level of pyrethroid resistance was detected in *An. funestus* populations in southern Mozambique. *An. funestus* outside Maputo province were still susceptible to pyrethroids. *An. funestus* from six localities also were resistant to carbamate insecticides propoxur and bendiocarb. Both pyrethroid and carbamate resistance occurred in five of these six localities. Mosquitoes from five of the localities with elevated p450 estimates, compared with the insecticide-susceptible Durban strain, were pyrethroid-resistant. The only exception to this trend was Mozal, which had elevated p450 estimates but full pyrethroid susceptibility by bioassay. The lack of cross-resistance between pyrethroids and DDT in Mozambican *An. funestus* suggests that a kdr-type target site resistance mechanism has not been selected. Low levels of insecticide-insensitive acetylcholinesterase, the target site for carbamates and organophosphates, were found in all populations tested. The high level of metabolically based pyrethroid resistance has implications for current malaria control programs in Mozambique.

Cocco P., Fadda D. & Melis M. (2006) "Reproductive outcomes following environmental exposure to DDT." *Reprod Toxicol.* **22**(1), 5-7. **Abstract:** We used
official statistics of births and stillbirths in 1945-1954 to evaluate reproductive outcomes in the general population following use of DDT during a 1946-1950 anti-malarial campaign in the Italian region of Sardinia. Due to the disruption of registration systems in the World War II years, data in the pre-DDT years were available only for 1945-1946. Such a short period of observation, and social conditions in the war and post-war years, do not allow exclusion of adverse effects of DDT on birth rate; however, we did not observe an effect. The stillbirth rate, infant mortality rate, and male/female ratio in newborns were apparently unaffected following widespread but focused use of DDT in Sardinia, Italy.

Dalvie M.A., Myers J.E., Lou Thompson M., Dyer S., Robins T.G., Omar S., Riebow J., Molekwa J., Kruger P. & Millar R. (2004) "The hormonal effects of long-term DDT exposure on malaria vector-control workers in Limpopo Province, South Africa." Environ Res. 96(1), 9-19. Abstract. DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] compounds, used in many developing countries, including South Africa, for the control of malaria vectors, have been shown to be endocrine disruptors in vitro and in vivo. The study hypothesis was that male malaria vector-control workers highly exposed to DDT in the past should demonstrate clinically significant exposure-related anti-androgenic and/or estrogenic effects that should be reflected in abnormalities in reproductive hormone levels. A cross-sectional study of 50 workers from three camps situated near the Malaria Control Center (MCC) in Tzaneen was performed. Tests included blood sampling before and after a gonadotropin-releasing hormone (GnRH) challenge (100 microg). Serum o’p’ and p’p’ isomers of DDE, DDT, and DDD and basal and post-GnRH challenge hormone levels, including luteinizing hormone, follicle-stimulating hormone, testosterone, sex hormone-binding globulin, estradiol (E2), and inhibin, were measured. The mean number of years worked at the MCC was 15.8+/-7.8 years and the mean serum DDT was 94.3+/-57.1 microg/g of lipid. Mean baseline E2 levels (62.4+/-29.9 pg/mL) exceeded the laboratory reference range. Associations between DDT exposure measures (years worked at the MCC and DDT compounds) and hormonal outcomes were weak and inconsistent. The most important finding was a positive relationship of baseline E2 and baseline testosterone with DDT compounds, especially with p’p’-DDT and -DDD. The strongest association found, adjusted for age and SHBG, was between baseline estradiol and p’p’-DDT (beta=1.14+/-0.33 pg/mL/microg/ g lipid, P=0.001, R2=0.31, n=46). An overall anti-androgenic mechanism best explains the results, but with a number of inconsistencies. Associations might be due to chance, as multiple comparisons were made. The results therefore do not suggest an overt anti-androgenic or estrogenic effect of long-term DDT exposure on hormone levels, but correlations do exist in a manner that is not understood.

Eskenazi B., Marks A.R., Bradman A., Fenster L., Johnson C., Barr D.B. & Jewell N.P. (2006) "In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children." Pediatrics. 118(1), 233-41. Abstract. OBJECTIVE: We investigated the relationship between prenatal exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene
(DDE) and neurodevelopment of Mexican farm-workers’ children in California. METHODS: Participants from the Center for the Health Assessment of Mothers and Children of Salinas study, a birth cohort study, included 360 singletons with maternal serum measures of p,p'-DDT, o,p'-DDT, and p,p'-DDE. Psychomotor development and mental development were assessed with the Bayley Scales of Infant Development at 6, 12, and 24 months. RESULTS: We found a approximately 2-point decrease in Psychomotor Developmental Index scores with each 10-fold increase in p,p'-DDT levels at 6 and 12 months (but not 24 months) and p,p'-DDE levels at 6 months only. We found no association with mental development at 6 months but a 2- to 3-point decrease in Mental Developmental Index scores for p,p'-DDT and o,p'-DDT at 12 and 24 months, corresponding to 7- to 10-point decreases across the exposure range. Even when mothers had substantial exposure, breastfeeding was usually associated positively with Bayley scale scores. CONCLUSIONS: Prenatal exposure to DDT, and to a lesser extent DDE, was associated with neurodevelopmental delays during early childhood, although breastfeeding was found to be beneficial even among women with high levels of exposure. Countries considering the use of DDT should weigh its benefit in eradicating malaria against the negative associations found in this first report on DDT and human neurodevelopment.

Farhang L., Weintraub J.M., Petreas M., Eskenazi B. & Bhatia R. (2005) "Association of DDT and DDE with birth weight and length of gestation in the Child Health and Development Studies, 1959-1967." Am J Epidemiol. 162(8), 717-25. Abstract. The pesticide p,p'-dichlorodiphenyltrichloroethane (DDT) and its persistent metabolite p,p'-dichlorodiphenyldichloroethylene (DDE) are associated with negative reproductive outcomes in animals. In humans, however, the findings are inconsistent. Using data from the Child Health and Development Studies, a longitudinal study of 20,754 pregnancies among San Francisco Bay Area women from 1959 to 1967, the authors examined the effects of maternal serum DDT and DDE concentrations on preterm birth, small-for-gestational-age birth, birth weight, and gestational age in 420 male subjects. Data were analyzed using multivariate logistic regression for preterm and small-for-gestational-age birth and linear regression for birth weight and gestational age. Median serum concentrations of DDE were 43 mug/liter (interquartile range: 32-57; range: 7-153) and of DDT were 11 mug/liter (interquartile range: 8-16; range: 3-72), several times higher than current US concentrations. The adjusted odds ratio for preterm birth was 1.28 (95% confidence interval (CI): 0.73, 2.23) for DDE and 0.94 (95% CI: 0.50, 1.78) for DDT. For small-for-gestational-age birth, the adjusted odds ratio was 0.75 (95% CI: 0.44, 1.26) for DDE and 0.69 (95% CI: 0.73, 1.27) for DDT; none of the study results achieved statistical significance. Given the persistence of DDT in the environment and its continuing role in malaria control, studies using more robust data should continue to assess this relation.

Abstract. This study from two districts of Orissa State which are endemic for Plasmodium falciparum transmitted by Anopheles fluviatilis and A. culicifacies investigated the impact of dichlorodiphenyl trichloroethane (DDT) indoor residual spraying, in view of the ongoing discussion on phasing out DDT in India. Based on their high annual parasite incidence and logistical considerations, 26 villages in Malkangiri and 28 in Koraput district were selected for DDT spraying. For comparison, six and four unsprayed villages were chosen from the same districts. In each district, the prevalence of malaria infection and incidence of malaria fever, indoor resting density and parous rate of the vectors, and their susceptibility to DDT were monitored in six and three villages selected randomly from the sprayed and unsprayed groups respectively. Anopheles fluviatilis was susceptible to DDT while A. culicifacies was resistant. DDT residual spraying with 1 g/m(2), was carried out in October-November 2001. Spraying 74-86% of human dwellings and 100% of cattle sheds brought down the indoor resting density of A. fluviatilis by 93-95%. This was associated with a significant reduction of incidence of malaria fever as well as prevalence of malaria infection from November to February in both districts. The spraying also seemed to impact on vector longevity, and a residual effect of DDT on the sprayed walls was observed up to 10-12 weeks despite re-plastering. Hence DDT spraying can still be an effective tool for controlling fluviatilis-transmitted malaria. Although this species is exophilic, its nocturnal resting behaviour facilitates its contact with the sprayed surfaces. DDT is still useful for residual spraying in India, particularly in areas where the vectors are endophilic and not resistant.

Herrera-Portugal C., Ochoa H., Franco-Sanchez G., Yanez L. & Diaz-Barriga F. (2005) "Environmental pathways of exposure to DDT for children living in a malarious area of Chiapas, Mexico." Environ Res. 99(2), 158-63. Abstract. Considering that DDT was used for control of malaria vectors in Mexico, and taking into account that the information regarding children in areas exposed to DDT is scarce, we started a research program for the assessment of health effects in children living in DDT sprayed areas. In this first report, we present information about pathways of exposure in two communities with a different history of exposure to DDT. Environmental pathways such as outdoor soils, indoor soils and household dust were assessed comparing a community highly exposed to DDT (HEC) and a community less exposed to DDT (LEC). Also in these communities, a cross-sectional study of 60 children (30 in each community) aged 6--12 years was conducted. Tests included a questionnaire and the measurement of whole blood DDT and DDE. Results show that in children living in the HEC, DDT and DDE mean blood levels were higher (15.9+/-8.2 and 58.2+/-29.2 microg/L) than in the LEC (1.9+/-3.6 and 9.2+/-5.7 microg/L) (P<0.01). Concentrations of DDT, DDE and DDD in indoor soil were higher in the HEC (10.3+/-10; 4.9+/-5.8; and 4.4+/-9.1mg/kg) than in the LEC (0.3+/-0.3; 0.04+/-0.06; and 0.03+/-0.04 mg/kg) (P<0.001). Similar results were obtained for outdoor soils; in the HEC, levels for DDT, DDE and DDD were 3.1+/-3.0; 1.0+/-0.8; and 0.3+/-0.2mg/kg; whereas levels in the LEC were 0.16+/-0.2; 0.02+/-0.03; and 0.02+/-0.03 mg/kg (P<0.001). High concentrations of DDT, DDE, and DDD were obtained in samples of indoor dust collected from the walls in the HEC.
De Jager C., Farias P., Barraza-Villarreal A., Avila M.H., Ayotte P., Dewailly E., Dombrowski C., Rousseau F., Sanchez V.D., Bailey J.L. (2006) "Reduced seminal parameters associated with environmental DDT exposure and p,p'-DDE concentrations in men in Chiapas, Mexico: a cross-sectional study." *J. Androl.* **27**(1), 16-27. Abstract. In response to mounting concerns about the endocrine-disrupting influence of environmental chemicals on human health, this epidemiological study was initiated to test the hypothesis that nonoccupational exposure to the estrogenic pesticide 1,1,1-trichloro-2,2-bis(chlorodiphenyl)ethane (DDT) affects male reproductive parameters. One hundred and sixteen men aged 27 years (SD = 8.2) living in malaria endemic-areas in Chiapas (Mexico), where DDT was sprayed until 2000, participated in a cross-sectional study. Semen analyses were conducted according to World Health Organization methods and a quality control program was followed. DDT exposure was defined as the level of blood plasma p,p'-dichlorodiphenyl dichloroethylene (DDE), the major metabolite of DDT. The p,p'-DDE concentration adjusted for total lipids was 100 times higher than that reported for nonexposed populations at 45 plus or minus 32 mug/g (mean +/- SD). Crude regression analysis showed that several sperm motion parameters, including the percentage of motile sperm, decreased with higher p,p'-DDE concentrations (beta = -8.38; P = .05 for squared motility), and the percentage of sperm with morphological tail defects increased with higher plasma p,p'-DDE concentration (beta = 0.003; P = .017). Insufficient sperm chromatin condensation was observed in 46.6% of participants, and the most severe category of incomplete DNA condensation was also positively correlated with p,p'-DDE concentration (r = .223; P = .044). Therefore, nonoccupational exposure to DDT, as assessed by plasma p,p'-DDE concentrations, is associated with poorer semen parameters in men, indicating adverse effects on testicular function and/or the regulation of reproductive hormones. Previously, a causal role of environmental toxicants in human male infertility has been lacking because observed effects have been the result of unusually high exposures, either occupationally or as a result of industrial accidents, resulting in unprecedented controversy (reviewed by Cheek & McLachlan “Environmental hormones and the male reproductive system.” *J Androl.* 1998;**19**,5). This is the first epidemiological study demonstrating effects after nonoccupational exposures to DDT. Based on these findings, the effect of DDT on male reproductive health should not be ignored.

Koepke R., Warner M., Petreas M., Cabria A., Danis R., Hernandez-Avila M. & Eskenazi B. (2004) "Serum DDT and DDE levels in pregnant women of Chiapas, Mexico." *Arch Environ Health.* **59**(11), 559-65. Abstract. The authors measured the main ingredients of technical DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl [p,p'-DDT]) and its principal metabolite, 1,1-dichloro-2,2-bis (p-
chlorophenyl)ethylene \([\text{p,p'}-\text{DDE}]\) in serum collected from 52 pregnant women in Tapachula, Chiapas, Mexico in 1998. The median lipid-adjusted serum levels for the women were 676 ng/g \(\text{p,p'}-\text{DDT}\) (range: 56-23,169 ng/g) and 4,843 ng/g \(\text{p,p'}-\text{DDE}\) (range: 113-41,964 ng/g). In regression analysis, serum DDT and DDE increased with age (test for trend, \(p = .022\)) but decreased with total lactation (test for trend, \(p < .001\)). Residence in a house that had ever been sprayed for malaria control was also related to serum DDT and DDE. This study provides evidence of high-level exposure to DDT and DDE among pregnant women living in Chiapas, Mexico, despite countrywide restrictions on its use at the time.


Maharaj R., Mthembu D.J. & Sharp B.L. (2005) "Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal." *S Afr Med J.* 95(11), 871-4.  Abstract. OBJECTIVES: To determine whether the re-introduction of DDT in KwaZulu-Natal had any effects on malaria transmission in the province. DESIGN, SETTING AND SUBJECTS: The 2000 malaria epidemic in KwaZulu-Natal has been attributed to pyrethroid-resistant anopheles mosquitoes in the area. Previous studies have shown that these mosquitoes are still susceptible to DDT. To determine whether DDT re-introduction had any impact on malaria transmission in KwaZulu-Natal, the following variables (pre- and post-epidemic) were investigated: (i) the number of reported cases; and (ii) the distribution of *Anopheles funestus* in relation to the insecticides sprayed. OUTCOME MEASURES: The notified malaria cases and the distribution of *A. funestus* were measured to determine the effects of DDT re-introduction on malaria transmission. RESULTS AND CONCLUSION: After DDT re-introduction, the number of malaria cases decreased to levels lower than those recorded before the epidemic. *A. funestus* appears to have been eradicated from the province. The combination of an effective insecticide and effective antimalarial drugs in KwaZulu-Natal has resulted in a 91% decline in the malaria incidence rate. Unfortunately the continued exclusive use of DDT within the malarious areas of the province is threatened by the emergence of insecticide resistance.


the effects and possible interactions between the public health interventions in use. METHODS: This study employed cross-sectional survey to collect data from households, community and health facilities on coverage and usage of Insecticide-Treated Nets (ITNs), Indoor Residual Spraying (IRS), larvicidal activities and malaria case management. Comparative data was obtained from a similar survey carried out in 2001. Data from the Health Management Information System (HMIS) and reports of the annual assessments by the National Malaria Control Programme was used to assess impact. Time series model (ARIMA) was used to assess association. RESULTS: In the period 2000-2004, approximately 874,000 ITNs were distributed and 13,109 health workers and community health agents were trained on malaria case management. In 2004, approximately 81% households owned at least one net, of which 73% were ITNs and 58.6% of children 0-5 years slept under a net. The proportion of malaria cases managed by community health agents rose from 50% in 1999 to 78% in 2004. IRS coverage increased with the combined amount of DDT and Malathion used rising from 6,444 kg, in 2000 to 43,491 kg, in 2004, increasing the population protected from 117,017 to 259,420. Drug resistance necessitated regimen change to chloroquine plus sulfadoxine-pyrimethamine. During the period, there was a steep decline in malaria morbidity and case fatality by 84% and 40% respectively. Malaria morbidity was strongly correlated to the numbers of ITNs distributed (beta = -0.125, p < 0.005) and the amount (kg) of DDT and Malathion used for IRS (beta = -2.352, p < 0.05). The correlation between malaria case fatality and ITNs, IRS, population protected and annual rainfall was not statistically significant. CONCLUSION: Eritrea has within 5 years attained key Roll Back Malaria targets. ITNs and IRS contributed most to reducing malaria morbidity.

Over M., Bakote'e B., Velayudhan R., Wilikai P. & Graves P.M. (2004) "Impregnated nets or DDT residual spraying? Field effectiveness of malaria prevention techniques in solomon islands, 1993-1999." Am J Trop Med Hyg. 71(2 Suppl), 214-23. Abstract. The incidence of malaria in Solomon Islands has been decreasing since 1992. The control program used a combination of methods including DDT residual house spraying and insecticide-treated mosquito nets. To determine how much each method contributed to malaria control, data were analyzed on monthly incidence and on control activities for 41 of 110 malaria zones over the same time period (January 1993 to August 1999). After correction for endogeneity, then spraying, insecticide treatment of nets, and education about malaria are all independently associated with reduction in incident cases of malaria or fever, while larviciding with temephos is not. The evidence suggests that although impregnated bed nets cannot entirely replace DDT spraying without substantial increase in incidence, their use permits reduced DDT spraying. The paper shows that non-experimental data can be used to infer causal links in epidemiology, provided that instrumental variables are available to correct for endogeneity.


Stuetz W., McGready R., Cho T., Prapamontol T., Biesalski H.K., Stepniewska K. & Nosten F. (2006) "Relation of DDT residues to plasma retinol, alpha-tocopherol, and beta-carotene during pregnancy and malaria infection: a case-control study in Karen women in northern Thailand." *Sci Total Environ.* 363(1-3), 78-86. Abstract. Populations living in endemic malaria areas maybe exposed simultaneously to DDT and malaria infection. DDT may impair status of vitamins, which are implicated in the immunity and pathophysiology of malaria. To explore possible interactions, DDT residues, retinol, alpha-tocopherol, beta-carotene and cholesterol were measured in plasma samples of malaria-infected pregnant women (cases, n=50) and age matched malaria-free controls (n=58). DDT residues were found in all samples: mean (sd) total DDT levels of 29.7 and 32.7 ng/ml in cases and controls, respectively. Mean (sd) p,p'-DDT was higher in the controls than the cases (13.5 vs. 9.5 ng/ml, p=0.006). Malaria infection was associated with lower mean (sd) plasma retinol (0.69 vs. 1.23 micromol/L) and cholesterol (2.62 vs. 3.48 mmol/L) compared to controls (p<0.001). Mean (sd) plasma alpha-tocopherol (7.65 vs. 15.58 micromol/L) and alpha-tocopherol/cholesterol ratio (2.3 vs. 6.7 micromol/L/mmol/L) were significantly lower among the controls (p<0.001). Mean (sd) plasma beta-carotene was low (<0.3 micromol/L) in both groups, but higher among malaria cases (0.19 vs. 0.15 micromol/L). Plasma retinol among the controls showed highly significant positive correlations with individual DDT compounds, particularly with p,p'-DDT (r=0.51, p<0.001). Plasma alpha-tocopherol and beta-carotene seemed not to be affected by DDT residues.