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# Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants

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**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 α-copaene, γ-cadinene, δ-cadinene , α-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 μg/ml.


*Keywords:* *Xylopia phloiodora*; *Xylopia aethiopica*; *Pachypodanthium confine*; *Hexalobus crispiflorus*; *Antidesma laciniatum*; Annonaceae; Euphorbiaceae; Malaria; *Plasmodium falciparum*; Essential oil

## 1. Introduction

Essential oils have demonstrated numerous biological actions, including antimicrobial activities (Carlton et al., 1992; Piccaglia et al., 1993; Buchbauer and Jirovetz, 1994; Aruna and Sivaramakrishnan 1996; Jazet Dongmo et al., 2002). These properties are likely due to multiple components of these complex mixtures and facilitated by ready diffusion across cell membranes. Individual oils may have hundreds of constituents, the principle components being terpenes (monoterpenes and sesquiterpenes) and their oxygenated derivatives. Other compounds include phenylpropenes and specific compounds containing sulfur or nitrogen.

Malaria is one of the most prevalent infections in the world. It constitutes one of the main causes of death in much of the tropics. Malaria is caused by parasites of the genus *Plasmodium*, after transmission by *Anopheles* mosquitoes. The most severe form of malaria is caused by *P. falciparum*, and this species is responsible for over a million deaths each year (Breman, 2001). *P. falciparum* is increasingly resistant to available antimalarial agents, and so the identification of new compounds that are active against the parasite is an urgent priority (Ridley, 2002).

Many studies have been carried out on the anti-plasmodial activities of non volatile compounds from various plant species with some encouraging results (Munoz et al., 2000; Hadi and Bremner, 2001; Bringmann, 2003). Of note, two of the most important antimalarial classes of drugs are natural products. Quinine

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and related compounds are alkaloids from *Cinchona* trees (Foley and Tilley, 1997). Artemisinin, from *Artemisia annua*, is the parent compound for a number of potent antimalarial drugs, including artesunate (Olliaro et al., 2001) and artemether (Pittler and Ernst, 1999). The antimalarial properties of essential oils have been little studied, although recent studies underlined the potential biological activities of these extracts against malaria parasites (Valentin et al., 1995; Lopes et al., 1999) and as insecticides against the mosquito vector (Moore and Lenglet, 2002). In this paper, we present the characterization of five essential oils extracted from Cameroonian medicinal plants and describe their activities against *P. falciparum* in culture.

### 1.1. Plant selection and uses

The plant species were selected based on previous interesting investigations on related plant families (Euphorbiaceae, Annonaceae) (Jenett-siems et al., 1999; Hadi and Bremner, 2001).

*Xylopia phloiodora* Mildbraed (Annonaceae) is a rain forest tree, with a thick yellowish bark, found in Central Africa and particularly in Cameroon, Central African Republic, Congo and Gabon (Le Thomas, 1969). *Pachypodanthium confine* Engler and Diels (Annonaceae) is a rain forest tree with a greyish bark, growing in Cameroon, Gabon, Congo and Angola. A decoction or maceration of the stem bark is used against body lice (Le Thomas, 1969). The results of two chemical investigations were reported on this species (Bevalot et al., 1977; Cave et al., 1973). *Antidesma laciniatum* Muell. Arg. var. *laciniatum* (Euphorbiaceae) is a small forest tree, which grows in Ivory Coast, Southern Nigeria, Cameroon, Equatorial Guinea and Congo. The bark is used powdered as an aphrodisiac (Hutchinson and Dalziel, 1958). *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) is an evergreen, aromatic tree, largely found in West, Central and Southern Africa. The fruits are mainly used in foods for their spicy properties, but also as a cough remedy, to relieve flatulence, and as a post-partum tonic. Other reported medicinal uses are for stomach ache, bronchitis, biliousness and dysentery (Iwu, 1993). The fruit has been shown to have antimicrobial action against gram positive and gram negative bacteria and *Candida albicans* (Boakye-Yiadom et al., 1977; Fournier et al., 1994; Tairu et al. 1999; Barminas et al., 1999; Iwu et al., 1999). Most studies were focused on the fruits of this species and very few on the other organs (Ngouela et al., 1998). *Hexalobus crispiflorus* A. Rich. (Annonaceae) is a rain forest tree with a tender yellowish bark growing across tropical Africa, from Sudan to Angola, Cameroon, Gabon and Senegal. A decoction of the bark is used as a purgative and emetic (Le Thomas, 1969).

## 2. Results and discussion

### 2.1. Characterization of essential oils

The essential 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 for *X. phloiodora, P. confine*, *A. laciniatum, X. aethiopica,* and *H. crispiflorus*. The results of the analyses of the essential oils are given in Table 1. The five evaluated essential oils contain mainly terpenoids (above 72%). Sesquiterpenes represent more than 87% of the oils of *H. crispiflorus* (99.5%), *P. confine* (88.0%), and *X. phloiodora* (87.8%) and 47.2 and 44.7% of those of *A. laciniatum* and *X. aethiopica*. The latter contains more than 55% monoterpenes, the majority of which are oxygenated derivatives (30.9%). Aromatic compounds were identified in the oils of *X. phloiodora* (2.55%), *P. confine* (0.64%) and *A. laciniatum* (27.3%), with benzyl benzoate being the most common component. Many sesquiterpenoid compounds were also identified in all five essential oils, including α-copaene, γ-cadinene, δ-cadinene, α-cadinol, spathulenol, and caryophyllene oxide.

### 2.2. Anti-plasmodial properties of essential oils

In initial experiments using microtiter culture plates, essential oils led to toxic effects on adjacent cultures, presumably due to volatile components. Therefore, subsequent experiments were performed with sealed flasks. Concentrations of essential oils above 0.6 mg/ml showed toxicity to erythrocytes, but anti-plasmodial activities were seen at much lower concentrations (Table 2).

All five essential oils were active against strain W2 of *P. falciparum* in culture. The most active essential oil was from *H. crispiflorus* ($IC_{50} = 2.0$ μg/ml) (Fig. 1). The potent activity of the oil of *H. crispiflorus* might be attributable to its high sesquiterpene content (99.5%). Sesquiterpenoids and their derivatives are credited with various biological actions, including antiasthmatic, antibacterial, antifungal, anti-inflammatory, and antineoplastic activities (Farnsworth and Bingel, 1977).

We have shown that five essential oils extracted from Cameroonian plants have anti-plasmodial activity, with one oil demonstrating particular potency. The large scale use of essential oils to cure various ailments suggests true biological activity, but little has yet been done on the evaluation of these oils against endemic tropical diseases. In the case of malaria, the physical properties of essential oils, including low density (~0.94 g/ml) and ready diffusion across cell membranes, might enhance targeting to intracellular malarial parasites.

In a recent study on antimalarial use of volatile plant extracts, Lopes et al. (1999) identified nerolidol (an acyclic oxygenated sesquiterpene) as one of the active

Table 1
Chemical composition of essential oils from five Cameroonian plants

| | | % | | | | |
|---|---|---|---|---|---|---|
| RI[a] | Compounds | *X. phloiodora* | *P. confine* | *A. laciniatum* | *X. aethiopica* | *H. crispiflorus* |
| | ***Monoterpene hydrocarbons*** | ***2.99*** | ***5.41*** | | ***24.29*** | ***0.25*** |
| 913 | α-Thujene | | 0.59 | | 0.61 | |
| 920 | α-Pinene | 0.58 | | | 4.05 | |
| 933 | Camphene | 1.38 | | | 4.87 | 0.13 |
| 955 | Sabinene | | 0.59 | | 0.46 | |
| 959 | β-Pinene | 0.68 | | | 10.07 | 0.12 |
| 999 | δ-3-Carene | | 1.1 | | | |
| 1006 | α-Terpinene | | 0.48 | | 0.43 | |
| 1011 | *p*-Cymene | 0.35 | 0.32 | | 1.72 | |
| 1038 | (*E*)-β-Ocimene | | 1.93 | | 1.13 | |
| 1058 | γ-Terpinene | | | | 0.58 | |
| 1068 | Terpinolene | | 0.4 | | 0.37 | |
| | ***Oxygenated monoterpenes*** | ***6.28*** | ***4.03*** | ***24.8*** | ***30.85*** | ***0.13*** |
| 1072 | Linalool | 0.31 | 0.22 | 9.4 | 1.58 | |
| 1095 | Nopinone | 0.65 | | | 2.53 | |
| 1096 | Fenchol | | 0.18 | | | |
| 1112 | (*E*)-Pinocarveol | 1.23 | 0.31 | | 5.42 | |
| 1117 | Camphor | | | | 1.4 | |
| 1147 | *p*-Cymen-8-ol | 1.58 | | | | |
| 1151 | Pinocarvone | | 3.16 | | 1.84 | |
| 1161 | Terpinen-4-ol | | 0.16 | | 0.49 | 0.13 |
| 1166 | Myrtenal | 0.28 | | | 2.85 | |
| 1172 | α-Terpineol | | | | 4.99 | |
| 1181 | Myrtenol | | | | 6.4 | |
| 1192 | Verbenone | 2.23 | | | 2.68 | |
| 1220 | Geraniol | | | 0.5 | | |
| 1256 | Thymol | 0.28 | | | | |
| 1263 | Bornyl acetate | | | | 0.67 | |
| 1345 | Geranyl acetate | | | 14.9 | | |
| | ***Sesquiterpene hydrocarbons*** | ***69.56*** | ***60.61*** | ***23.4*** | ***33.1*** | ***75.54*** |
| 1334 | δ-Elemene | 3.29 | 0.53 | | 0.44 | 0.21 |
| 1347 | α-Cubebene | | 0.52 | | 1.04 | 0.36 |
| 1356 | α-Ylangene | | | | 5.32 | 0.33 |
| 1361 | α-Copaene | 0.53 | 7.06 | 2.2 | 4.07 | 13.27 |
| 1372 | β-Bourbonene | | | 0.5 | | |
| 1373 | Cyclosativene | 2.07 | | | | |
| 1374 | β-Elemene | 0.58 | 0.73 | | 1.34 | 1.92 |
| 1387 | Cyperene | 0.34 | 15.54 | | 3.95 | 11.53 |
| 1397 | α-Cedrene | | 6.02 | | | |
| 1401 | Isocaryophyllene | | | | | 0.75 |
| 1404 | α-Gurjunene | | 1.86 | | 0.64 | |
| 1413 | β-Caryophyllene | | 0.28 | 5.2 | 1.67 | 1.33 |
| 1414 | β-Copaene | | | 0.3 | | |
| 1423 | (*E*)-α-Bergamotene | 0.46 | 0.18 | | | |
| 1447 | Aromadendrene | | | | | 1.08 |
| 1453 | α-Humulene | | 5.04 | 2.1 | 1.09 | 1.76 |
| 1455 | Alloaromadendrene | | | | | 8.52 |
| 1455 | γ-Muurolene | | 0.35 | 0.7 | 2.64 | 1.93 |
| 1458 | *epi*-bicyclo-Sesquiphellandrene | 3 | | | | |
| 1461 | Germacrene D | 1.02 | 0.84 | 8.5 | 0.94 | 2.62 |
| 1472 | α-Muurolene | | | 1.5 | 1.84 | 1.29 |
| 1492 | γ-Cadinene | 11.27 | 3.51 | 0.3 | | 2.53 |
| 1494 | Bicyclogermacrene | 1.43 | 2.41 | | | |
| 1497 | β-Selinene | | 0.28 | | | 2.2 |
| 1499 | (*E*,*E*)-α-Farnesene | | 2.66 | 0.5 | 0.56 | |
| 1501 | α-Selinene | 21.92 | 1.41 | | 0.82 | 3.37 |
| 1505 | δ-Cadinene | 15.11 | 8.06 | 1.3 | 4.3 | 10.07 |
| 1506 | Cadina-1,4-diene | | | | 0.67 | |
| 1507 | Calacorene | 0.89 | | | 0.84 | 7.82 |

(*continued on next page*)

Table 1 (*continued*)

| RI[a] | Compounds | % | | | | |
|---|---|---|---|---|---|---|
| | | *X. phloiodora* | *P. confine* | *A. laciniatum* | *X. aethiopica* | *H. crispiflorus* |
| 1513 | (*Z*)-Calamenene | | 2.32 | | 0.93 | 1.09 |
| 1519 | α-Cadinene | | 1.01 | 0.3 | | |
| 1636 | Cadalene | 7.65 | | | | 1.56 |
| | ***Oxygenated sesquiterpenes*** | ***18.24*** | ***27.34*** | ***23.8*** | ***11.56*** | ***23.91*** |
| 1518 | Elemol | 2.04 | 1.24 | | 1.09 | |
| 1537 | (*E*)-Nerolidol | 0.64 | 2.42 | | | 2.82 |
| 1543 | Germacrene D-4-ol | | | 0.4 | | |
| 1551 | Spathulenol | 1.02 | 2.16 | 1.4 | 6.33 | 1.97 |
| 1555 | Caryophyllene oxide | 5.07 | 7.24 | 8.5 | 1.99 | 2.54 |
| 1567 | Fonenol | 0.76 | | | | |
| 1568 | γ-Eudesmol | | | | | 0.99 |
| 1574 | Globulol | 1.93 | 2.38 | | | |
| 1578 | Humulene oxide | 0.68 | 3.17 | 3.5 | | 1.38 |
| 1595 | Cubenol | | 3.23 | | | |
| 1597 | T-Muurolol + torreyol | 3.7 | 2.1 | | | |
| 1602 | *epi*-α-Cadinol | 0.95 | 1.25 | 0.2 | 1 | 7.34 |
| 1607 | *epi*-α-Muurolol | | | 2.5 | | |
| 1611 | 1,10-di-*epi*-Cubenol | | | | | 1.3 |
| 1619 | β-Eudesmol | | | | 1.15 | 1.08 |
| 1635 | α-Muurolol | 0.58 | 0.64 | 1 | | |
| 1638 | α-Cadinol | 0.5 | 1.16 | 3 | | 1.41 |
| 1643 | α-Eudesmol | | | | | 1.61 |
| 1680 | (*E*,*E*)-Farnesol | | | 2 | | |
| 1682 | Farnesol | 0.37 | 0.35 | | | |
| 1687 | *epi*-α-Bisabolol | | | | | 1.47 |
| 1789 | (*E*,*E*)-Farnesyl acetate | | | 1.3 | | |
| | ***Aromatic compounds*** | ***2.55*** | ***2.42*** | ***27.3*** | | |
| 989 | *p*-Methyl anisole | | | 2.1 | | |
| 1062 | Methyl benzoate | | | 0.5 | | |
| 1127 | Benzyl acetate | | | 1.5 | | |
| 1138 | Ethyl benzoate | 0.25 | | | | |
| 1253 | (*E*)-Anethole | | | 0.5 | | |
| 1400 | (*E*)-Cinnamyl acetate | | | 0.6 | | |
| 1474 | Eugenyl acetate | | 1.78 | | | |
| 1512 | Methoxy cinnamaldehyde | 1.47 | | | | |
| 1621 | 2,4,5-Trimethoxy-styrene | | 0.43 | | | |
| 1719 | Benzyl benzoate | 0.83 | 0.21 | 19.1 | | |
| 1826 | Benzyl salicylate | | | 3 | | |

[a] (Retention indices = Kovats indices); components were identified based on RI and GC-MS (gas chromatography coupled with mass spectrometry) and listed according to their order of elution on DB1 (50 m); % = percent peak area of essential oil constituents. *X. phloiodora* = *Xylopia phloiodora* (stem bark); *P. confine* = *Pachypodanthium confine* (stem bark); *A. laciniatum* = *Antidesma laciniatum* (leaves); *X. aethiopica* = *Xylopia aethiopica* (stem bark); *H. crispiflorus* = *Hexalobus crispiflorus* (stem bark).

Table 2
Anti-plasmodial activity of the essential oils

| Plant species (organ) | $IC_{50}$[b] (μg/ml) |
|---|---|
| *Xylopia phloiodora* (stem bark) | 17.9 |
| *Pachypodanthium confine* (stem bark) | 16.6 |
| *Antidesma laciniatum* (leaves) | 29.4 |
| *Xylopia aethiopica* (stem bark) | 17.8 |
| *Hexalobus crispiflorus* (stem bark) | 2.0 |
| Chloroquine phosphate | 30.4 nM |

[b] Concentration that killed 50% of parasites relative to negative control.

principles. Another recent study suggested the presence of an active isoprenoid pathway for biosynthesis of isoprenic chains of coenzyme Q in *P. falciparum* (De Macedo et al., 2002). Parasites treated with nerolidol showed decreased ability to synthesize coenzyme Q in all intraerythrocytic stages. In our study, nerolidol was identified in the essential oils of *X. phloiodora* (0.64%), *P. confine* (2.42%), and *H. crispiflorus* (2.82%), but not in the two other essential oils. Thus, this compound may have contributed part, but certainly not all of the demonstrated antimalarial activity.

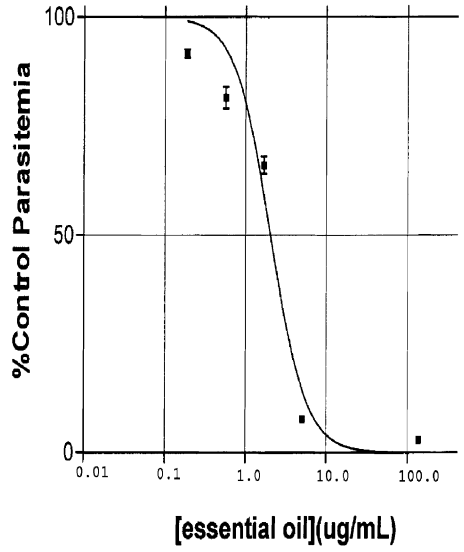


Fig. 1. Different dilutions of the essential oil were incubated with cultured W2-strain *P. falciparum* parasites for 48 h, parasites were fixed and stained, and parasitemias of treated and control cultures were determined. Results are means, compared to untreated controls, from three experiments. Error bars represent standard deviations of results.

## 3. Concluding remarks

Although the specific components of the studied essential oils that elicit anti-plasmodial activity remain unclear, our data suggest that the oils offer new possibilities for antimalarial chemotherapy. Although essential oils are necessarily difficult to study and difficult to utilize as drugs, they may offer a unique means of discovering new effective antimalarial drugs from plants with medicinal uses in endemic countries. Important goals will be to identify the active components of essential oils with antimalarial activity and to determine the mechanisms by which these compounds exert their biological activities.

## 4. Experimental

### 4.1. Collection and extraction of plant material

The leaves of *A. laciniatum* were collected in Mount Kalla, near Yaoundé, Cameroon in March 1999. The stem bark of *X. phloiodora, X. aethiopica, P. confine*, and *H. crispiflorus* were collected in Mbalmayo, a Yaoundé suburb, in April 2001. The plant samples were identified and voucher specimens deposited at the National Herbarium (Yaoundé), with the following respective identification numbers: 28732/SRF/Cam, 32062/SRF/Cam, 59700 HNC1, 038/SRF/Cam, and 42175/HNC. Air-dried leaves and bark were ground using a blender. Batches of 500 g of plant material were subjected to hydro-distillation for 3 h using a Clevenger type apparatus (Fekam Boyom et al., 2003). The resulting essential oils were dried over anhydrous sodium sulfate.

### 4.2. Analysis of the chemical composition of the essential oils

The oils were analysed on a Varian CP-3380 gas chromatograph with flame ionization detectors fitted with a fused silica capillary column (30 m × 0.25 mm i.d.) coated with DB1 phase, film thickness 0.25 μm; temperature program 50–200 °C at 5 °C/min, injector temperature 220 °C, detector temperature 250 °C, carrier gas $N_2$ at 0.8 ml/min. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes and the percentage compositions were obtained directly from a Shimadzu C-R4A recorder by electronic integration measurements (Fekam Boyom et al., 2003). GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with a $HP_1$ fused silica column (30 m × 0.25 mm; film thickness 0.25 μm) and interfaced with a quadrupole detector (Model 5970). Column temperature was programmed from 70–200 °C at 10 °C/min; injector temperature was 220 °C. Helium was used as carrier gas at a flow rate of 0.6 ml/min; the mass spectrometer was operated at 70 eV. The identification of the constituents was assigned on the basis of comparison of their retention indices (Kovats indices) and their mass spectra with those given in the literature (McLafferty and Stauffer 1989; Adams, 1995; Joulain and König, 1998).

### 4.3. Evaluation of anti-plasmodial activity

*P. falciparum* strain W2, which is resistant to chloroquine and other antimalarials (Singh and Rosenthal, 2001), was cultured in sealed flasks at 37 °C, in a 3% $O_2$, 5% $CO_2$ and 91% $N_2$ atmosphere in RPMI 1640, 25 mM HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 2% hematocrit. Parasites were synchronized in the ring stage by serial treatment with 5% sorbitol (Sigma) (Lambros and Vanderberg, 1979) and studied at 1% parasitemia.

Essential oils were prepared as 10% (v/v) stock solutions in DMSO, diluted as needed for individual experiments, and tested in triplicate. The oil stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture was thereafter added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1 μM chloroquine phosphate (Sigma). Cultures were incubated at 37 °C for 48 h (one parasite erythrocytic life cycle). Parasites at ring stage were thereafter fixed

by replacing the serum medium by an equal volume of 1% formaldehyde in PBS. Aliquots (50 μl) of each culture were then added to 5 ml round-bottom polystyrene tubes containing 0.5 ml 0.1% Triton X-100 and 1 nM YOYO nuclear dye (Molecular Probes) in PBS. Parasitemias of treated and control cultures were compared using a Becton-Dickinson FACSort flow cytometer to count nucleated (parasitized) erythrocytes. Data acquisition was performed using CellQuest software. These data were normalized to percent control activity and 50% inhibitory concentrations ($IC_{50}$s) calculated using Prism 3.0 software (GraphPad) with data fitted by non linear regression to the variable slope sigmoidal dose-response formula $y = 100/[1 + 10^{(\log IC_{50} - x)H}]$, where $H$ is the hill coefficient or slope factor (Singh and Rosenthal, 2001).

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