PharmacologyOnLine

August 30, 2015

Archives • 2015 • vol.2 • 1-5

# CHEMICAL COMPOSITION AND IN VITRO ANTIPLASMODIAL ACTIVITY OF ESSENTIAL OILS OF LEAVES AND FLOWERS OF *ALPINIA ZERUMBET* GROWN IN CUBA.

Mendiola, J.[1*]; Pino, J.A.[2]; Fernández-Calienes, A.[1]; Mendoza, D. [1]; Herrera, P.[3]

[1]Department of Parasitology and Cellular Cultures Lab, Institute of Tropical Medicine "Pedro Kourí", Havana, Cuba
[2]Food Industry Research Institute, Carretera al Guatao km 3½, Havana, Cuba
[3]Institute of Ecology and Systematic, Havana, Cuba

[*]mendiola@ipk.sld.cu

## Abstract

*Alpinia zerumbet* (Pers.) B.L. Burtt & R. M. Smith was recommended to treat malaria fevers before eradication in Cuba. The chemical analysis identified 68 and 75 volatile compounds of the essential oils isolated from leaves and flowers of *A. zerumbet* grown in Cuba, respectively. Major components of leaf volatile oil were terpinen-4-ol (19.0%), caryophyllene oxide (18.0%), β-eudesmol (8.9%), β-caryophyllene (7.6%) and (*E*)-nerolidol (5.4%); while for flower essential oil were viridiflorol (32.2%), terpinen-4-ol (14.1%), caryophyllene oxide (6.9%) and 1,8 cineole (5.0%). *A. zerumbet* leaf and flower oil exhibited mild antiplasmodial activity with values of concentrations causing 50% inhibition of *in vitro* growth of *Plasmodium berghei* ($IC_{50}$) of 71.4 ± 1.5 µg/mL and 66.2 ± 6.4 µg/mL, respectively. Cytotoxicity to human lung fibroblast cells of the MRC-5 line was relatively low. Future studies of these essential oils in order to support the use of this plant as traditional remedy would be worthwhile.

**Keywords**: *Alpinia zerumbet*, antiplasmodial, cytotoxicity, essential oils, traditional medicine.

## Introduction

*Alpinia zerumbet* (Pers.) B.L. Burtt & R. M. Smith is an aromatic plant found in tropical and sub-tropical regions of the world [1]. This species is known popularly as ''colonia'' in Cuba [1,2] where it is useful in traditional medicine: flowers and leaves decoctions are used in the treatment of common cold; flowers and rhizomes are diuretics, while external applications of leaves decoctions are useful for skin diseases and headaches [2]; in particular the mixed decoction of leaves of *A. zerumbet* and *Citrus x aurantiifolia* (Christm.) Swing was recommended for bath or oral use to treat fevers produced by malaria [3] Certainly, *A. zerumbet* essential oils from leaves, seeds and fruits exhibited pharmacological activities which have been associated to the treatment of intestinal disorders, hypertension and cardiovascular diseases. Consequently, bioactivity has been related to their diverse chemical compositions [4,5,6].

In the perspective to find out new crude essential oils with antimalarial activity, the present work was aimed to describe the chemical composition and to explore the activity of the leaf essential oil (LEO) and flower essential oil (FEO) isolated of *A. zerumbet* grown in Cuba against *in vitro* growth of *Plasmodium berghei* and a normal human lung cell line.

## Methods

### *Plant material*

Leaves and flowers of *A. zerumbet* were collected in January 2010, from plants grown in the National Botanical Garden (NBG) in Havana, Cuba. The species was identified by Dr. Pedro Herrera from the Cuban Institute of Ecology and Systematic and a voucher specimen was deposited at the Herbarium of NBG (HFC 87096). Fresh leaves (200g) and flowers (100g) were submitted to hydrodistillation in a Clevenger-type apparatus for 2 h. At the end of each distillation, the oils were collected, dried with anhydrous $Na_2SO_4$, measured, and transferred to glass flasks that were filled to the top and kept at a temperature of −18 °C for further analysis. Analyses were made by duplicate. Yields were calculated according to the weights of oils and plant material before distillation.

### *Analysis of the essential oils*

Analyses were performed on a Konik 4000A instrument (Barcelona, Spain) equipped with a HP-5ms fused silica column (25 m x 0.25 mm i.d., film thickness 0.25 µm), split injection 1:10, and flame ionization detection. Injector and detector temperatures were at 220 ºC and 250 ºC. The oven temperature was held at 70 ºC for 2 min and then raised to 250 ºC at 4 ºC/min and held for 10 min. The carrier gas was $H_2$ at 1 mL/min. Samples were injected by splitting and the split ratio was 1:20. The lineal retention indices (RI) were obtained from gas chromatography (GC) by logarithmic interpolation between bracketing a homologous series of *n*-alkanes used as standards. Peak areas were measured by electronic integration using the EZ Chrom Chromatography Data System 6.07 program (Scientific Software, Inc., Florida). The relative amount of the individual components was based on the peak areas.

Gas chromatography/ Mass spectrometry (GC/MS) analysis was performed on a Shimadzu 17A (Tokyo, Japan) gas chromatograph coupled to a Shimadzu QP-5000 high performance quadrupole mass selective detector. The GC was fitted with a HP-5ms fused silica column (25m x 0.25 mm i.d., film thickness 0.25 µm). The GC operating conditions were identical with those described above except that He was used as carrier gas. The MS operating conditions were: ionization potential 70 eV with scan mass range of 35 – 400 *m/z* and ion source temperature at 250 ºC.

Compounds were identified by computer search using digital libraries of mass spectral data (NIST 02, Wiley 275, Adams 2001, Palisade 600, and Flavorlib homemade library) and by comparison of their retention indices of either reference substances or literature values [7], relative to $C_8$-$C_{32}$ *n*-alkane series in a temperature programmed run.

### *Parasites*

After thawing of *Plasmodium berghei* ANKA stocks (gently donated by the Laboratory of Microbiology, Parasitology and Hygiene of University of Antwerp, Belgium), the parasite population was maintained *in vivo* by serial transfer of parasitized erythrocytes from infected to naive Balb/C mice. All experiments were carried out in accordance with international guidelines for animal care in biomedical research [8] and were described in the project 0903022 approved by the Ethical Committee of the Institute of Tropical Medicine "Pedro Kourí".

### *In vitro antimalarial activity*

Short-term *in vitro* cultures of *P. berghei* ANKA blood stages were performed as described before [9]. Ring forms and young trophozoites were obtained from donor mice and exposed to serial dilutions of dimethylsulfoxide (DMSO) dissolved volatile oils from 200 µg/mL to 12.5 µg/mL in complete culture

medium. Duplicate wells of 96-well culture plates were incubated overnight (16–20 h) at 37 °C and analysed as previously specified [10]. Schizonts numbers were determined by light microscopy in test and control wells (0.5 % of DMSO). The antimalarial activity was expressed as 50 % inhibitory concentration ($IC_{50}$), defined as the concentration of essential oil that induces 50 % reduction of development to schizonts, which was calculated according to reported methodology [11] for data of three independent experiments. Chloroquine phosphate (Sigma, USA) was used as reference to ascertain assays performance [12].

***Mammalian cell line cytotoxicity assay***

MRC-5 (ATCC CCL-171), a human diploid lung fibroblast line, was grown in minimal essential medium (Gibco-BRL, USA) supplemented with 10 % FBS (CELLect GOLD, MP Biomedicals, USA), *L*-glutamine and non-essential amino acids. As preincubation conditions, 1 x $10^4$ cells were seeded per well in 96-well culture plates and left to grow for 24 h at 37 °C and 5 % $CO_2$. The formed monolayers were treated with two-fold, serial dilutions of essential oils in duplicates and allowed to grow for additional 72 h. The cytotoxicity of each sample was determined with the MTT (dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide, Sigma, USA) assay according to Mossman (1983) [13]. The cytotoxicity was expressed as 50 % cytotoxic concentration ($CC_{50}$), defined as such that induces killing of 50% of live cells in control wells (prepared with 0.5 % of DMSO). They were calculated from data of three independent experiments and fitted to the non-linear regression function for sigmoidal dose-response plots using GraphPad Prism 5.0 program.

## Results

A yield of 0.25% (v/m) of LEO was obtained by hydrodistillation of the fresh leaves of *A. zerumbet* while a yield of 0.20% (v/m) of FEO was produced.

Constituents identified in analyses of LEO and FEO are listed in the table. In total, 68 and 75 volatile compounds were identified in LEO and FEO, respectively (>99% of total composition). In LEO, oxygenated sesquiterpenes (50.7%) and oxygenated monoterpenes (34.1%) were the most abundant chemical class of compounds identified followed by sesquiterpene hydrocarbons (14.1%) and monoterpene hydrocarbons (<0.1%). The major monoterpene found was terpinen-4-ol (19.0%). Among the sesquiterpenes, caryophyllene oxide (18.0%), β-eudesmol (8.9%), β-caryophyllene (7.6%) and (E)-nerolidol (5.4%) were present in appreciable quantities. In FEO, oxygenated sesquiterpenes (56.4%) and oxygenated monoterpenes (31.5%) were also predominant, whereas lower proportions were assigned to sesquiterpene hydrocarbons (7.3%) and monoterpene hydrocarbons (3.3%). The major sesquiterpenes were viridiflorol (32.2%) and caryophyllene oxide (6.9%); among monoterpenes, terpinen-4-ol (14.1%) and 1,8 cineole (5.0%) were most important.

The *A. zerumbet* LEO inhibited parasite growth *in vitro* at $IC_{50}$=71.4 ± 1.5 µg/mL while FEO inhibited at $IC_{50}$ = 66.2 ± 6.4 µg/mL. These values indicate a mild antiplasmodial activity of both volatile oils [14], so they were less active than chloroquine ($IC_{50}$ = 17.6 ng/mL). LEO affected human lung fibroblast cells with $CC_{50}$ = 118.5 µg/mL in a range 88.6 - 158.4 µg/mL as 95% confidence interval while FEO had $CC_{50}$ = 95.5 µg/mL in a range 82.0 – 111 µg/mL as confidence interval. $CC_{50}$ from LEO and FEO were values only 1.66 and 1.44 higher than the antiplasmodial $IC_{50}$, respectively.

## Discussion

According to previous published data [4,5,6], the results of the present study related to *A. zerumbet* LEO are slightly different, because the LEO from plants grown in Cuba had higher amounts of sesquiterpenes in comparison with these reports. FEO of this plant has not been analyzed previously, to the best of our knowledge.

As $CC_{50}$ ≥ 100 µg/mL has been considered as no significantly toxic for other studies with the MRC-5 line[15] and previous research did not report high toxicity for pharmacological studies of *A. zerumbet* leaf essential oils with hamsters and mice[4,5], the *in vivo* antimalarial activity of LEO and FEO should be evaluated.

Among components identified in this study, only linalool and *trans*-nerolidol have been reported to possess antiplasmodial activity [16]. Therefore, the observed LEO and FEO antiplasmodial activities suggest the result of the combination of individual known active components with other structural different compounds present in LEO and FEO which should be additionally estimated.

In conclusion, the current investigation reports on particular composition enriched in sesquiterpenes of LEO and FEO obtained of *A. zerumbet* grown in Cuba, as well as its mild antimalarial activity, which should be a focus for future studies of these natural products in order to support the use of this plant as traditional remedy.


ISSN: 1827-8620

## References


1. Acevedo-Rodríguez, P., Strong, M.T., Catalogue of Seed Plants of the West Indies. Smithsonian Contributions to Botany 2012;98:1-1192.
2. Roig, J.T., Plantas medicinales, aromáticas o venenosas de Cuba. La Habana: Editorial Científico-Técnica (Spanish); 1974:324-325.
3. Hernández, J., Volpato, G., Herbal mixtures in the traditional medicine of Eastern Cuba. J Ethnopharmacol 2004;90:293–316.
4. Lin, L.Y., Peng, C.C., Liang, Y.J., et al., *Alpinia zerumbet* potentially elevates high-density lipoprotein cholesterol level in hamsters. J Agric Food Chem 2008;56:4435–4443.
5. Murakami, S., Matsuura, M., Satou, T., et al., Effects of the essential oil from leaves of *Alpinia zerumbet* on behavioral alterations in mice. Nat Prod Commun 2009;4:129-132.
6. Shen, X.C., Tao, L., Li, W.K., et al., Evidence-based antioxidant activity of the essential oil from Fructus *A. zerumbet* on cultured human umbilical vein endothelial cells' injury induced by ox-LDL. BMC Complement Altern Med 2012;12:174-183.
7. Adams, R.P., Identification of essential oil components by gas chromatography quadrupole mass spectroscopy. Carol Stream : Allured Publishing Corp 2001:455.
8. European Union (EU). Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official J EU 2010;L276:33-79.
9. Janse, C.J., Waters, A.P., *Plasmodium berghei*: the application of cultivation and purification techniques to molecular studies of malaria parasites. Parasitol Today 1995;11:138-143.
10. Mendiola, J., Regalado, E.L., Díaz-García, A., et al., In vitro antiplasmodial activity, cytotoxicity and chemical profiles of sponge species of Cuban coasts. Nat Prod Res 2014;28:312-317.
11. Huber, W., Koella, J.C., A comparison of three methods of estimating $EC_{50}$ in studies of drug resistance of malaria parasites. Acta Trop 1993; 55:257-261.
12. Orjuela-Sánchez, P., Duggan, E., Frangos, J.A., et al., A lactate dehydrogenase ELISA-based assay for the in vitro determination of *Plasmodium berghei* sensitivity to anti-malarial drugs. Malaria J 2012;11:366.
13. Mossman, T.J., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. J Immunol Methods 1983;65:55-63.
14. Cos, P., Vlietinck, A.J., Vanden Berghe, D., et al., Anti-infective potential of natural products: How to develop a stronger in vitro "proof-of-concept". J Ethnopharmacol 2006;106:290-302.
15. Lay, M.M., Karsani, S.A., Mohajer, S., et al., Phytochemical constituents, nutritional values, phenolics, flavonols, flavonoids, antioxidant and cytotoxicity studies on *Phaleria macrocarpa* (Scheff.) Boerl fruits. BMC Complement Altern Med 2014;14:152-163.
16. Rodrigues Goulart, H., Kimura, E.A., Peres, V.J., et al., Terpenes arrest parasitic development and inhibit biosynthesis of isoprenoids in *Plasmodium falciparum*. Antimicrob Agents Ch 2004;48:2502-2509.



ISSN: 1827-8620

**Table 1.** Chemical composition of *A. zerumbet* essential oils from leaves and flowers.

| Compound | RI* | LEO (%) | FEO (%) | Compound | RI* | LEO (%) | FEO (%) |
|---|---|---|---|---|---|---|---|
| 2-heptanol | 898 | nd[#] | 0.1 | eugenol | 1359 | tr | tr |
| α-thujene | 930 | nd | tr[¶] | neryl acetate | 1362 | nd | tr |
| α-pinene | 939 | tr | tr | α-ylangene | 1371 | tr | tr |
| camphene | 954 | nd | tr | α-copaene | 1374 | tr | tr |
| benzaldehyde | 960 | tr | tr | methyl (*E*)-cinnamate | 1377 | tr | 0.1 |
| **sabinene** | 975 | tr | **1.9** | geranyl acetate | 1381 | nd | 0.1 |
| myrcene | 991 | nd | 0.1 | daucene | 1385 | 0.1 | 0.2 |
| α-phellandrene | 1003 | tr | tr | β-elemene | 1391 | 0.1 | tr |
| α-terpinene | 1017 | tr | 0.3 | **β-caryophyllene** | 1419 | **7.6** | **3.0** |
| *p*-cymene | 1024 | tr | 0.1 | 4-phenyl-2-butyl acetate | 1423 | 0.1 | nd |
| limonene | 1028 | tr | tr | *trans*-α-bergamotene | 1435 | 0.3 | 0.1 |
| β-phellandrene | 1030 | tr | nd | α-guaiene | 1440 | 0.1 | 0.1 |
| **1,8-cineole** | 1033 | nd | **5.0** | γ-patchoulene | 1445 | 0.3 | 0.1 |
| 2-heptyl acetate | 1044 | tr | tr | **α-humulene** | 1455 | **1.8** | 0.5 |
| γ-terpinene | 1060 | tr | 0.8 | allo-aromadendrene | 1460 | tr | 0.3 |
| ***cis*-sabinene hydrate** | 1070 | 0.5 | **1.1** | β-selinene | 1490 | 0.7 | 0.8 |
| terpinolene | 1089 | tr | 0.2 | α-selinene | 1496 | nd | 0.5 |
| **linalool** | 1097 | nd | **3.2** | α-muurolene | 1500 | nd | 0.3 |
| ***trans*-sabinene hydrate** | 1098 | **4.1** | nd | β-biabolene | 1506 | nd | 0.1 |
| ***cis*-2-*p*-menthen-1-ol** | 1122 | **1.3** | 0.7 | **γ-cadinene** | 1514 | **1.8** | 0.7 |
| ***trans*-2-*p*-menthen-1-ol** | 1141 | **1.6** | 0.6 | 7-*epi*-α-selinene | 1522 | 0.0 | nd |
| camphor | 1146 | nd | 0.1 | ***trans*-calamenene** | 1529 | **1.3** | 0.5 |
| borneol | 1169 | nd | 0.1 | (*Z*)-nerolidol | 1533 | nd | tr |
| **terpinen-4-ol** | 1177 | **19.0** | **14.1** | α-cadinene | 1539 | tr | 0.1 |
| cryptone | 1186 | tr | tr | α-calacorene | 1545 | tr | tr |
| **α-terpineol** | 1189 | **4.3** | **3.6** | **elemol** | 1550 | **1.5** | tr |
| *cis*-piperitol | 1193 | nd | 0.2 | **(*E*)-nerolidol** | 1563 | **5.4** | nd |
| myrtenol | 1196 | 0.7 | nd | **ledol** | 1569 | nd | **1.4** |
| *trans*-piperitol | 1208 | 1.5 | 0.4 | **caryophyllene oxide** | 1583 | **18.0** | **6.9** |
| *trans*-carveol | 1217 | 0.0 | tr | humulene epoxide I | 1590 | tr | nd |
| β-citronellol | 1226 | 0.0 | 0.2 | **viridiflorol** | 1595 | **1.0** | **32.2** |
| cuminaldehyde | 1242 | 0.0 | 0.4 | **guaiol** | 1600 | nd | **3.3** |
| geraniol | 1250 | 0.1 | 0.1 | **palustrol** | 1605 | nd | **4.4** |
| piperitone | 1253 | 0.1 | nd | **humulene epoxide II** | 1610 | **3.3** | **2.1** |
| carvenone | 1258 | 0.1 | tr | ***epi*-α-muurolol** | 1638 | **1.2** | nd |
| geranial | 1266 | tr | nd | **hinesol** | 1642 | **3.7** | **1.3** |
| (*E*)-cinnamaldehyde | 1270 | tr | tr | **α-muurolol** | 1646 | **3.3** | 0.1 |
| bornyl acetate | 1285 | 0.5 | 0.5 | **β-eudesmol** | 1651 | **8.9** | **1.4** |
| thymol | 1288 | tr | tr | **α-cadinol** | 1656 | 0.9 | **3.0** |
| *p*-cymen-7-ol | 1292 | tr | nd | **14-hydroxy-9-*epi*-(*E*)-caryophyllene** | 1670 | **2.4** | nd |
| carvacrol | 1299 | 0.2 | tr | (*Z*)-α-santalol | 1675 | 0.7 | 0.2 |
| 4-vinylguaiacol | 1323 | tr | tr | α-bisabolol | 1686 | 0.4 | nd |
| *p*-mentha-1,4-dien-7-ol | 1333 | 0.3 | 0.1 | (*Z*)-β-santalol | 1716 | tr | nd |
| **α-terpinyl acetate** | 1349 | nd | **1.4** | phytol | 2112 | 0.4 | tr |
| citronellyl acetate | 1353 | nd | tr | | | | |

*Retention index in HP-5ms column; [#]not detected; [¶]trace (<0.1%); Components at more than 1% are in bold.


ISSN: 1827-8620