**Pharmacogn. Commn. 2017; 7(1) 41-46**
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

*Original Article*

# Antiplasmodial Activities of the Stem bark Extract and Compounds of *Zanthoxylum gilletii* (De wild) P.G. Waterman

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## ABSTRACT

**Introduction:** Multidrug resistance remains a major obstacle hindering successful antimalarial chemotherapy. In the current study, 50% methanol in dichloromethane extract and six compounds from the stem bark of *Zanthoxylum gilletii* were explored for their antiplasmodial potential against three strains of *Plasmodium falciparum*. **Materials and Methods:** The extract was obtained by cold percolation using 50 % MeOH in $CH_2Cl_2$ and the antiplasmodial activities were assayed using a non–radioactive Malaria SYBR Green I assay to determine a concentration that inhibits growth of 50% of parasites in culture ($IC_{50}$). **Results and Discussion:** Chromatographic separation of the crude extract yielded six known compounds including: one lignan, sesamin (**1**), an alkamide, fagaramide (**2**), three benzo [*c*] phenanthridine alkaloids, 8–acetonyldihydrochelerythrine (**3**), dihydrochelerythine (**4**), norchelerythrine (**5**) and one pentacyclic triterpenoid, lupeol (**6**). The extract and sesamin (**1**) showed promising antiplasmodial activities against the chloroquine resistant (W2), chloroquine sensitive (D6) and 3D7 strains of *P. falciparum*, with $IC_{50}$ values of 2.52, 1.48 and 1.43 µg/mL and 5.4, 9.1 and 8.3 µM, respectively. To the best of our knowledge, this is the first report on antiplasmodial activities of the stem bark extract (50% MeOH in $CH_2Cl_2$) and compound **1**–**3** and **6**. Furthermore, three of the isolated compounds; **1**, **3**, **6** are reported from this species for the first time. **Conclusion:** The good antiplasmodial activities exhibited by the stem bark of *Z. gilletii* against three different strains of *P. falciparum* may be attributed to the presence of **1**-**3** exhibiting good activities against all strains of *P. falciparum*.

**Key words:** 8–Acetonyldihydrochelerythrine, Sesamin, Antimalarial potencies, *Zanthoxylum gilletii.*

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**DOI :** 10.5530/pc.2017.1.6

## INTRODUCTION

Malaria is a parasitic disease affecting approximately 350–500 million people worldwide with 1.1 million deaths yearly. In many parts of the world the parasites have developed resistance to a number of antimalarials including the most widely used malaria treatment; chloroquine and its derivatives, and the current drugs of choice, artemisin and combinations. This therefore calls for urgent need to search for newer antimalarial principles, ideally with different modes of action to curb the resistance problem. Plants commonly used in traditional medicine are good candidates as sources of active anti-malarial principles.[1] In Kenyan traditional medicine, plants belonging to the genus *Zanthoxylum* are extensively used to manage a number of ailments including malaria.

*Zanthoxylum* (Rutaceae) with approximately 250 species, grows as shrubs or trees and are distributed in the tropics, sub tropics and the temperate regions of the world.[2] Kenya is endowed with seven species found in moist or dry forests, or in the thickets near the sea. These include: *Z. holstzianum (*Engl.) Waterman, *Z. usamarense* (Engl.) Kokwaro, *Z. chalybeum* (Engl.) var, *chalybeum*, *Z. gilletii* (De wild) P.G. Waterman, *Z. mildbraedii* (Engl.) P.G. Waterman, *Z. paracantum* (Mildbr) Kokwaro and *Z. rubescens* Planch. Ex Hook.f.[3] There are several traditional uses reported from members of the genus *Zanthoxylum*. Some have even served as raw materials in pharmaceutical and cosmetic practice.[4] In Africa members of the family Rubiaceae including *Zanthoxylum* species have been used for the management of malaria in different countries.[5] Furthermore, the leaves and the root bark of *Zanthoxylum* species have been used for the treatment of various diseases including: for the treatment of stomachaches, toothaches, coughs, urinary infections rheumatism, leprous ulcerations and venereal diseases.[2,6] Previous scientific research has shown that plants belonging to the genus *Zanthoxylum* have good bioactivities including: larvicidal, analgesics, anthelminthic, anti-viral, antioxidant anti–fungal, anti–biotic, anti-inflammatory and cytotoxicity.[2,7-10] *Z. gilletii* is a valued forest tree that grows naturally, but is commercially planted in Western Kenya for timber and medicinal properties.[11] The Luhya community that is a major habitat of this region, and populations in Mont Koupé region in Cameroun, use the stem bark of this plant in traditional anti-malarial preparations.[12-14] Previous studies have revealed that different part of *Zanthoxylum* species used in Kenyan traditional medicine exhibited anti-plasmodial activities similar to those of *Z. gilletii*. For example, the methanol extracts of the leaves of *Z. chalybeum* showed anti-plasmodial activities with an $EC_{50}$ value of 8.10 (5.89–11.12) µg/ml, which was more potenthan the positive control, chloroquine diphosphate, with an $EC_{50}$ value of 25.33 (17.07–37.60) µg/ml.[15] In earlier studies, the water and methanol extracts of the root bark of *Z. chalybeum* exhibited interesting antiplasmodial activities against both the choroquine sensitive and resistant strains of *Plasmodium falciparum*, with $IC_{50}$ values of < 6 µg/mL. These results are consistent with results observed for the methanol extract of the stem bark of *Z. gilletii*.[16,17] In a separate study, the water extract of the stem bark of *Z. usambarensis* which is commonly used in Kenyan traditional medicine, exhibited good anti-plasmodial activities against *P. knowlesi* with an $IC_{50}$ value of 6.04 ± 0.11 µg/ml.[18] Similarly, the methanol extract of the stem bark of this plant was more potent, with $IC_{50}$

values < 6 µg/ml compared with the aqueous extract which showed $IC_{50}$ values between 6 and 15 µg/ml against both chloroquine–sensitive and resistant *P. falciparum* isolates.[19] Furthermore, the stem bark extracts have shown interesting *vitro* anti-plasmodial activities in previous studies exhibiting $IC_{50}$>5 µg/mL.[20,21]

The consistencies in anti-plasmodial activities of these extracts from different *Zanthoxylum* species may be attributed to the presence of similar phytochemical profiles in different parts of these plants. The bark of *Z. gilletii* are also used to manage stomachache, joint pain, toothache, fever, rheumatism, venereal infections and for washing wounds.[11] Various phytochemical studies carried out on some *Zanthoxylum* species have revealed the presence of alkaloids of various skeletal types including: benzophenanthridine,[22-25] protoberberine,[26] bishordeninyl,[27] aporphine[28-30] amides,[31-35] coumarins,[4,34,36-38] lignans[39-44] as common secondary metabolites which also have chemotaxonomic importance to the genus. Metabolites such as flavonoids,[33,45-47] sterols and terpenes have also been isolated from plants from this genus.[48-52] Other isolated included: volatile oils, vanillic acid and hydroxyl benzoic acid.[8,9,51] These compounds are most probably responsible for the activities of plants in this genus.

In our continued search for biologically active anti-plasmodial compounds from Kenyan medicinal plants, we report the isolation of six known compounds from the 50% MeOH in $CH_2Cl_2$ extract of the stem bark of *Z. gilletii* together with their anti-plasmodial activities.

## MATERIALS AND METHODS

### General Experimental Procedures

Merck silica gel 60 (70–230 mesh) and Sephadex LH–20 were used as stationary phases for column chromatography (CC). Preparative thin layer chromatography (PTLC) (1.0 mm, 20 x 20 cm) were prepared using Merck silica gel 60 ($PF_{254+366}$); factory made analytical aluminium TLC plates (silica gel 60 $F_{254,}$ Merck) were used to monitor the purity of the isolates by visualizing the spots under UV light at 254 or 366 nm for UV active compounds, followed by placing the plate in an iodine tank and spraying with Dragendorff's reagent for both the non–UV active and alkaloid compound tests, respectively. The $^1H$ and $^{13}C$ NMR spectra were recorded using Varian–Mercury 200 MHz and Bruker–Avance 500 and 600 MHz spectrometers. The Homo Nuclear Correlation Spectroscopy (COSY), Hetero Nuclear Single Quantum Coherence (HSQC) and Hetero nuclear Multiple Bond Connectivity (HMBC) spectra were obtained using standard Bruker software. Chemical shifts were measured in ppm relative to the internal standard tetramethylsilane (TMS). The major solvents used for chromatography were *n*–hexane ($n$–$C_6H_{14}$), dichloromethane ($CH_2Cl_2$), ethyl acetate (EtOAc) and methanol (MeOH).

#### Plant material

The stem bark of *Zanthoxylum gilletii* was collected from Kakamega Forest, a tropical rainforest in Western Kenya (approx. 60 km from Kisumu city) in February, 2014. The plant was identified by Mr. Patrick Mutiso, a technologist, at the University of Nairobi Herbarium, School of Biological Sciences, where a voucher specimen (Masinde–010/2014/09) is deposited.

#### Extraction and isolation

The stem bark of *Zanthoxylum gilletii* (3.8 kg) was air dried under shade, pulverized into fine powder using a Willy mill at the Department of Chemistry, University of Nairobi. The ground plant material were then exhaustively extracted by soaking in a mixture of 3 litres of MeOH and 3 litres of $CH_2Cl_2$ for a period of 72 h at room temperature and then filtering. The filtrate was concentrated *in vacuo* using rotary evaporator and combined to give a yellowish and partly oily extract (7.8% of the pulverized material). The extract obtained using 50% MeOH in $CH_2Cl_2$ (100 g) was adsorbed onto an equal amount of silica gel (100 g) and loaded onto 500 g of silica gel column packed under 100% $n$–$C_6H_{14}$. The column was eluted serially with solvent systems of increasing polarity, initially with 2 % EtOAc in $n$–$C_6H_{14}$ and subsequently with increasing amounts of EtOAc upto 100%. A total of 160 fractions (200 ml each) were collected, concentrated *in vacuo* in a rotatory evaporator and their compound profiles monitored using analytical TLC plates. Similar fractions were combined, eventually giving a total of fourteen fractions. Lupeol (**6**, 1.4 g) was isolated as white crystalline powder, [mp 214-215 ºC (Lit. (212-214 ºC); $[\square]_D$ +20.4º (c = 0.56, $CH_2Cl_2$), Lit. $[\square]^{25}_D$ +25.7 (c 0.70 in $CHCl_3$),),[53] from the fractions of the main column eluted with 2% EtOAcin $n$–$C_6H_{14}$, while the fractions of the main column eluted with 3% EtOAcin $n$–$C_6H_{14}$ crystallized in the conical flask. The crystals were filtered out *in vacuo* using a Buchner funnel and washed severally using 90% $CH_2Cl_2$ in $n$–$C_6H_{14}$ and dried in open air, yielding only 20 mg of dihydrochelerythrine (**4**). The fractions of the major column eluted with 4% EtOAcin $n$–$C_6H_{14}$ were combined, solvent removed *in vacuo* using a rotatory evaporator and re-crystallized using 60% $CH_2Cl_2$ in $n$–$C_6H_{14}$ resulting to off–white amorphous solids of the lignin, sesamin (**1**, 2 g) [mp 120-121 ºC (Lit. (122-123 ºC); $[\square]_D$+27.6º (c = 0.56, $CH_2Cl_2$), Lit. $[\square]_D$ +66.4º (c 2 in $CHCl_3$).[54] The mother liquor was re-crystallized from 80% $CH_2Cl_2$ in $n$–$C_6H_{14}$, filtered and dried yielding, 8–acetonyldihydrochelerythrine (**3**, 25 mg). The fractions of the main column eluted with 5–8% were combined and solvent removed *in vacuo* with a rotatory evaporator and loaded onto a Sephadex LH 20 column leading to isolation of white amorphous solids of norchelerythrine (**5,** 15 mg) and an aromatic amide, fagaramide (**2,** 3g)

#### In vitro anti-plasmodial assay

The extracts and compounds were assayed using a non–radioactive Malaria SYBR Green I assay technique[55] to determine a concentration that inhibits growth of 50% of parasites in culture ($IC_{50}$). In this method, three different *Plasmodium falciparum* strains *vis* chloroquine sensitive Sierra Leone I (D6), chloroquine sensitive 3D7 and chloroquine–resistant Indochina I (W2), were grown as described by Trager and Jensen (1976);[56] with minor modifications.[57] Drugs, extracts and compounds were dissolved in 99.5% dimethylsulfoxide (DMSO) (Sigma–Aldrich) and diluted in complete Roswell Park Memorial Institute 1640 series of Cell Culture Media (RPMI 1640) enriched with human serum. The RPMI 1640 medium was prepared accordingly as described by Akala *et al*. (2011).[58] Briefly, the basic culture medium was prepared from RPMI 1640 powder (10.4 g; Invitrogen, Inc. augmented with 2 g glucose (Sigma Inc.) and 5.95 g of HEPES (Sigma Inc.), dissolved to homogeneity in 1 L of de–ionized water and sterilized with a 0.2 µM filter. Complete RPMI 1640 media, used for all parasite culture and drug dilutions, consisted of basic RPMI 1640 media with 10% (v/v) human ABO pooled plasma, 3.2% (v/v) sodium bicarbonate (Thermo Fisher Scientific Inc.) and 4 µg/mL hypoxanthine (Sigma Inc.). Drug preparation entailed two–fold serial dilutions of chloroquine (1.953–1000 ng/mL), mefloquine (0.488–250 ng/mL) and test sample (97.7–50,000 ng/mL) were prepared on a 96–well plate, such that the final proportion of DMSO was equal to or less than 0.0875%. The culture–adapted *Plasmodium falciparum* at 2% hematocrit and 1% parasitemia, were then added on to the plate containing dose range of drugs and incubated in a gas mixture (5% $CO_2$, 5% $O_2$, and 90% $N_2$) at 37 °C. The assay was terminated 72 h later by freezing at −80 °C and parasite growth inhibition was quantified as described by Johnson *et al*. (2007)[57] and the results presented as mean $IC_{50}$ ± SD.

## RESULTS AND DISCUSSION

#### Structure elucidation

In search for more effective antimalarial principles from Kenyan ethnomedicinal flora, chromatographic separation of the crude extract of stem

**Table 1:** *In-vitro* $IC_{50}$ values of the crude extract and compounds from *Z. gilletii* against W2, D6 and 3D7 strains of *P. falcipurum*

| Samples Tested | $IC_{50}$ in µg/ml (µM) | | |
|---|---|---|---|
| | W2 strain (CQ resistant) | D6 (CQ sensitive) | 3D7 (Chloroquine sensitive) |
| Stem bark extract (50% MeOH in $CH_2Cl_2$) | 2.52 ± 0.4 | 1.48 ± 0.3 | 1.43 ± 0.2 |
| Sesamine (**1**) | 1.92 ± 0.5 (5.4) | 3.23 ± 0.7 (9.1) | 2.94 ± 0.6 (8.3) |
| Fagaramide (**2**) | 15.15 ± 1.1 (61.3) | 7.73 ± 1.5 (31.3) | 7.72 ± 0.9 (31.2) |
| 8-Acetonyldihydrochelerythrine (**3**) | 4.02 ± .7 (9.92) | 4.06 ± .9 (10.0) | 3.37 ± 1.0 (8.31) |
| Lupeol (**6**) | 32.95 ± 8.1 (77.3) | - | 4.52 ± 1.7 (99.7) |
| Chloroquine | 0. 04 ± 0.02 | 0.001 ± 0.0001 | 0.004 ± 0.002 |
| Mefloquine | 0.001 ± 0.0005 | - | 0.01 ± 0.001 |

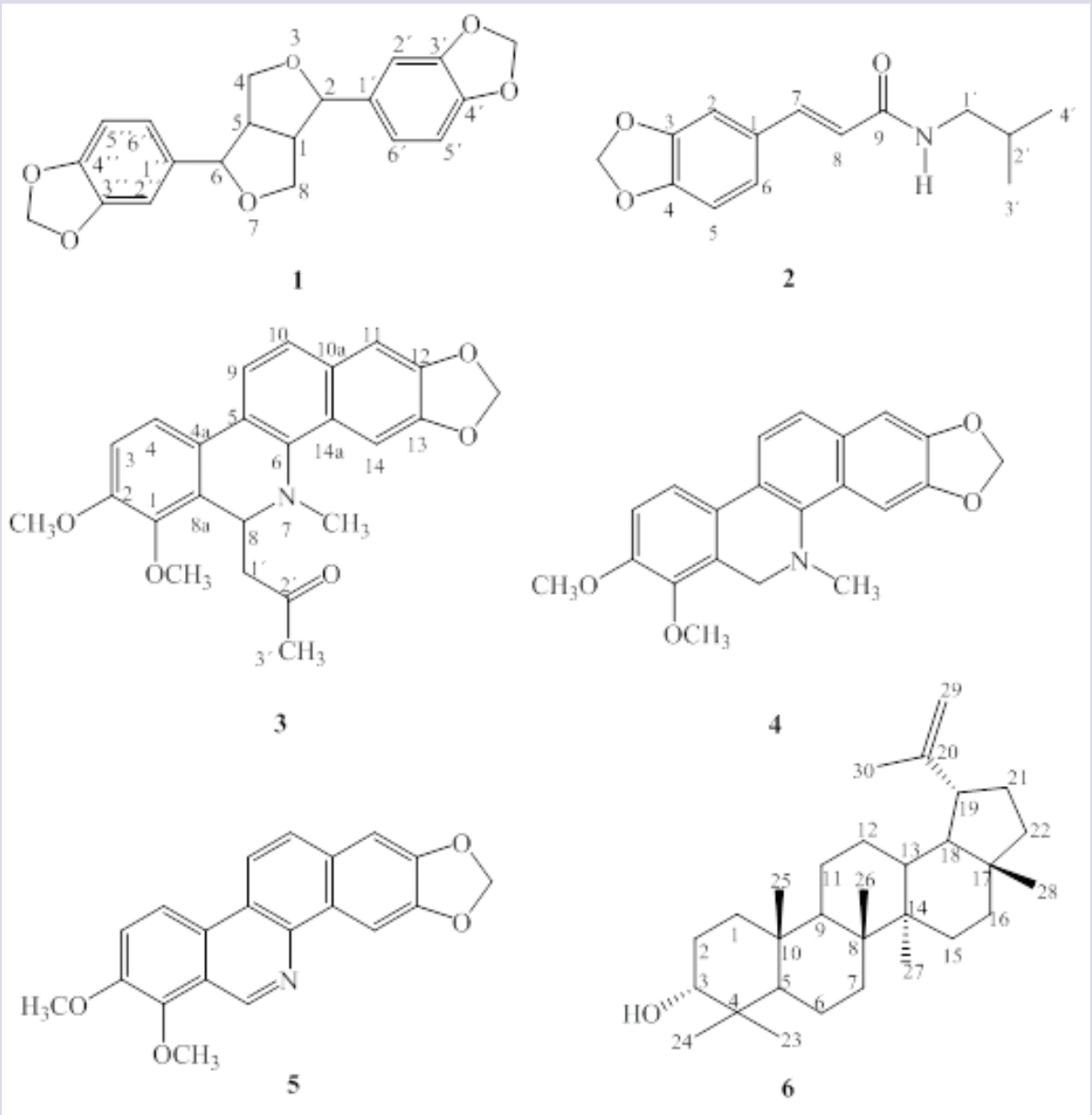



**Figure 1:** Compounds **1-6** from *Zanthoxylum gilletii*.

bark of *Z. gilletii* was obtained using 50% MeOH in $CH_2Cl_2$. This led to the isolation of one lignan; sesamin (**1**),[23-24] an amide; fagaramide (**2**),[59] three benzophenanthridine alkaloids; 8–acetonyldihydrochelerythrine (**3**),[10,60-63] dihydrochelerythine (**4**),[10,59] norchelerythrine (**5**)[64] and one terpenoid; lupeol (**6**).[65-67] Three of the compounds; **1, 3, 6** are reported for the first time from this species. The structures of these compounds were identified using UV, MS, ID and 2D NMR spectroscopy and supported by literature values. Sesamin (**1**), fagaramide (**2**) 8–acetonyldihydrochelerythrine (**3**) and lupeol (**6**) were isolated in sufficient amounts to be investigated for *in vitro* antimalarial potencies.

### *Bioactivity results*

The crude and some pure compounds obtained from the stem bark of *Zanthoxylum gilletii* were tested for their antiplasmodial activities. From the criteria of evaluation of *in vitro* anti-plasmodial activities of natural products, pure compounds are considered to be inactive when they exhibit an $IC_{50}$>200 μM, low activity from 100–200 μM, moderate activity from 20–100 μM, good activity from 1–20 μM and excellent/potent anti-plasmodial activity <1 μM.[68] Similarly, crude extract activities are categorized as having good activity when they show an $IC_{50}$<10 μg/mL; moderate activity from 10–50 μg/mL; low activity from 50–100 μg/mL and inactive >100 μg/mL.[69]

From the above evaluation criteria the MeOH in $CH_2Cl_2$ (1:1) extract of the stem bark of *Z. gilletii* showed good antiplasmodial activities against the chloroquine resistant (W2), chloroquine sensitive (D6) and 3D7 strains of *P. falciparum*, with $IC_{50}$ values of 2.52, 1.48 and 1.43 μg/ml, respectively. The compounds tested showed good to moderate antiplasmodial activities with the lignan, sesamin (**1**) exhibiting the highest activities with $IC_{50}$ values of 5.4, 9.1 and 8.3 μM against W2, D6 and 3D7 strains of *P. falciparum*. Lignans, synthesized in nature by oxidative dimerization of various phenylpropanoid, are known to have diverse biological activity profiles.[70-71] These include: antitumor, antimitotic, antiviral, antimicrobial, antinociceptive antiulcerogenic activities, inhibition of certain enzymes, and carrageenan induced edema in mice.[72-75] Earlier studies have revealed the antiplasmodial potential of different classes of lignans including: furfuran against the chloroquine–resistant *P. falciparum*[76] and aryltetralone against *P. berghei*.[77] Other classes of lignans including: tetrahydrofuran lignans, neolignans, dibenzylbutanelignan and a coumarinolignan also demonstrated varying degrees of antimalarial activities against mainly *P. falciparum* with tetrahydrofuran–type sesquilignans revealing significant activities.[78-82] Compound **3** was also active against the three strains of the malaria parasite tested with $IC_{50}$ value of 9.92, 10.0 and 8.31 μM against W2, D6 and 3D7 strains, respectively. Fagaramide (**2**) showed moderate activity with $IC_{50}$ values of 31.3 and 31.2 μM against D6 and 3D7 but less active against W2 strain of *P. falciparum* with $IC_{50}$ value of 61.3 μM. Surprisingly, lupeol (**6**) exhibited incomparable $IC_{50}$ values of 77.3 μM against W2 and 9.96 against 3D7 strain. The antiplasmodial potency of **2** is consistent with those exhibited by similar alkamides from the leaves of *Z. syncarpum* (the racemic form of the syncarpamide), which showed good antiplasmodial activity, with $IC_{50}$ values of 4.2 and 6.1 μM against D6 and W2 strains of *P. falciparum*, respectively.[35] Surprisingly, lupeol (**6**) exhibited incomparable $IC_{50}$ values of 32.95 against W2 and 4.25 μM against 3D7 strain (Table 1).

## CONCLUSION

To the best of our knowledge, this is the first report on antiplasmodial activities of the stem bark extract (50% MeOH in $CH_2Cl_2$) and compounds **1**–**3** and **6**. Furthermore, three of the isolated compounds; **1**, **3**, **6** are reported here for the first time from this species. The good antiplasmodial activities exhibited by the stem bark of *Z. gilletii* against three different strains of *P. falciparum* may validate its traditional use to manage malaria and related ailments. The pharmacological properties of the stem bark of this plant may be attributed to the presence of the lignan; sesamin (**1**), an alkamide; fagaramide (**2**) and a benzophenanthridine alkaloid; 8–acetonyldihydrochelerythrine (**3**) which exhibited good activities against all strains of *P. falciparum*. The compounds from this plant should be re–isolated in order to carry out structure activity relationship studies of compounds **3**, **4** and **5** with similar skeletal structures and substitution pattern except for the presence of a keto group attached to a methyl group in **3** speculated to be responsible for activity. Furthermore, related alkaloids from *Z. monophyllum* displayed strong antibacterial activities against both the drug sensitive *Aspergillus fumigatus* and methicillin–resistant *Staphylococcus aureus* (MRSA).[83] the alkaloids isolated from *Z. gilletii* should also be subjected to antimicrobial activities against the two strains of bacteria to determine their antimicrobial potential.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

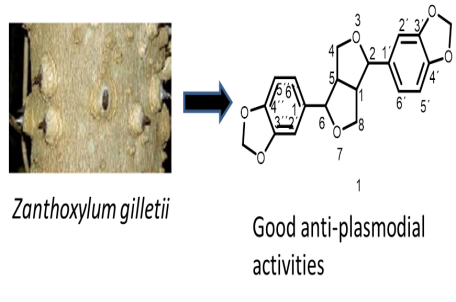
## REFERENCES


1. Bero J, Frédérich M, Quetin-Leclercq J. Antimalarial compounds isolated from plants used in traditional medicine. J Pharm Pharmacol. 2009;61(11):1401-33. http://dx.doi.org/10.1211/jpp.61.11.0001 ; PMid:19903367
2. Negi JS, Bisht V, Bhandari AK, Singh P, Sundriyal RC. Chemical constituents and biological activities of the genus Zanthoxylum. A review, Afr. J Pure Appl Chem. 2011;5(12):412-6.
3. Beentje H. Kenya trees shrubs and lianas, Nairobi Kenya: National Museums of Kenya,1994.
4. Bafi–Yeboa NFA, Arnason JT, Baker J, Smith ML. Antifungal constituents of Northern prickly ash Zanthoxylum americanum Mill. Phytomedicine. 2005;12(5):370-7. http://dx.doi.org/10.1016/j.phymed.2003.12.005 ; PMid:15957372
5. Iwu MM. African medicinal plants in the search for new drugs based on ethnobotanical leads, Ciba Foundation Symposium, 1994;185:116-126; discussion 126–129. PMid:7736850
6. Dharani N, Rukunga G, Yenesew A, Mbora A, Mwaura L, Dawson I, *et al.* Common antimalarial trees and shrubs of East Africa.A Description of Species and a Guide to Cultivation and Conservation Through Use. 2010;73-6.
7. Tatsadjieu LN, EssiaNgang JJ, Ngassoum MB, Etoa F–X. Antibacterial and antifungal activity of Xylopia aethiopica Monodora myristica Zanthoxylum xanthoxyloides and Zanthoxylum leprieurii from Cameroon. Fitoterapia. 2003;74(5):469-72. http://dx.doi.org/10.1016/S0367-326X(03)00067-4
8. Ngassoum MB, Essia–Ngang JJ, Tatsadjieu LN, Jirovetz L, Buchbauer G, Adjoudji O. Antimicrobial study of essential oils of Ocimum gratissimum leaves and Zanthoxylum xanthoxyloides fruits from Cameroon. Fitoterapia. 2003;74(3):284-7. http://dx.doi.org/10.1016/S0367-326X(03)00035-2
9. Paik SY, Koh KH, Beak SM, Paek SH, Kim JA. The essential oils from Zanthoxylum schinifolium pericarp induce apoptosis of HepG2 human hepatoma cells through increased production of reactive oxygen species. Biol Pharm Bull. 2005;8(5):802-7. http://dx.doi.org/10.1248/bpb.28.802
10. Luo X, Pedro L, Milic V, Mulhovo S, Duarte A, Duarte N, *et al.* Antibacterial benzofuranneolignans and benzophenanthridine alkaloids from the roots of Zanthoxylum capense. Planta Med. 2012;78(2):148-53. http://dx.doi.org/10.1055/s-0031-1280289 ; PMid:22002848
11. Kokwaro JO. Medicinal Plants of East Africa 3rd Edition–Nairobi: University of Nairobi Press 2009;256.
12. Nyunja ARC, Onyango JC, Erwin B. The Kakamega Forest Medicinal Plant Resources and their Utilization by the Adjacent Luhya Community. Int J Trop Med. 2009;3:82-90.
13. Guédé NZ, N'guessan K, Dibié TE, Grellier P. Ethnopharmacological study of plants used to treat malaria in traditional medicine by Bete Populations of Issia (Côte d'Ivoire). J Pharm Sci Res. 2010;2(4):216-27.
14. Japheth OO, Josphat MC, John VM. Chemical composition, and larvicidal activity of Zanthoxylum gilletii essential oil against Anopheles gambiae. Afri J Biotechnol. 2014;13(21):2175-80. http://dx.doi.org/10.5897/AJB2014.13711
15. Bbosa GS, Mwebaza N, Lubega A, Musisi N, Kyegombe DB, Ntale M. Antiplasmodial Activity of Leaf Extracts of Zanthoxylum chalybeum Engl. Brit J Pharm Res. 2014;4(6):705-13. http://dx.doi.org/10.9734/BJPR/2014/6528
16. Rukunga GM, Gathirwa JW, Omar SA, Muregi FW, Muthaura CN, Kirira PG, *et al.* Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. J Ethnopharmacol. 2009;121(2):282-5. http://dx.doi.org/10.1016/j.jep.2008.10.033; PMid:19041710
17. Muganga R, Angenot L, Tits M, and Frederich M. Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. J Ethnopharmacol. 2010;128(1):52-7. http://dx.doi.org/10.1016/j.jep.2009.12.023; PMid:20035853

18. Were P, Kinyanjui P, Gicheru MM, Mwangi E, Ozwara HS. Prophylactic and curative activities of extracts from Warburgia ugandensis Sprague (Canellaceae) and Zanthoxylum usambarense (Engl,) Kokwaro (Rutaceae) against Plasmodium knowlesi and Plasmodium berghei. J Ethnopharmacol. 2010;130(1):158-62. http://dx.doi.org/10.1016/j.jep.2010.04.034 ; PMid:20435133
19. Kirira PG, Rukunga GM, Wanyonyi AW, Muregi FM, Gathirwa JW, Muthaura CN, *et al*. Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi Districts of Kenya. J Ethnopharmacol. 2006;106(3):403-7 http://dx.doi.org/10.1016/j.jep.2006.01.017 ; PMid:16530996
20. Atindehou KK, Schmid C, Brun R, Koné MW, Traore D. Antitrypanosomal and antiplasmodial activity of medicinal plants from Côte d'Ivoire. J Ethnopharmacol. 2004;90(2):221-7. http://dx.doi.org/10.1016/j.jep.2003.09.032 ; PMid:15013184
21. Zirihi GN, Grellier P, Guédé–Guina F, Bodo B, Mambu L. Isolation characterization and antiplasmodial activity of steroidal alkaloids from Funtumia elastica (Preuss) Stapf. Bioorgan Med Chem Letters. 2005;15(10):2637-40. http://dx.doi.org/10.1016/j.bmcl.2005.03.021 ; PMid:15863333
22. De Moura NF, Ribeiro HB, Machado ECS, Ethur EM, Zanatta N, Morel AF. Benzophenanthridine alkaloids from Zanthoxylum rhoifolium. Phytochemistry. 1997;46(8):1443-6.doi:10.1016/S0031–9422(97)00498–6. http://dx.doi.org/10.1016/S0031-9422(97)00498-6
23. Nissanka AP, Karunaratne V, Bandara BR, Kumar V, Nakanishi T, Nishi M, *et al*. Antimicrobial alkaloids from Zanthoxylum tetraspermum and caudatum. Phytochemistry. 2001;56(8):857–61. http://dx.doi.org/10.1016/S0031-9422(00)00402-7
24. Tane P, Wabo HK, Connolly JD. A new benzophenanthridine alkaloid from Zanthoxylum buesgenii. Fitoterapia. 2005;76(7):656-60. http://dx.doi.org/10.1016/j.fitote.2005.08.018; PMid:16242267
25. Mansoor TA, Borralho PM, Luo X, Mulhovo S, Rodrigues CMP, Ferreira M–JU. Apoptosis inducing activity of benzophenanthridine–type alkaloids and 2–arylbenzofuranneolignans in HCT116 colon carcinoma cells. Phytomedicine. 2013;20(10):923-9. doi:10.1016/j.phymed.2013.03.026. http://dx.doi.org/10.1016/j.phymed.2013.03.026
26. Jiang Hu W-DZ, Alkaloids from Zanthoxylum nitidum (Roxb,) DC. Biochem. Syst Ecol. 2007;35(2):114-7, doi:10.1016/j.bse.2006.10.001. http://dx.doi.org/10.1016/j.bse.2006.10.001
27. Marcos M, Villaverde MC, Riguera R, Castedo L, Stermitz FR. Zanthoxylum coriaceum alkaloids related to bishordeninyl terpenes. Phytochemistry. 1990;29(7):2315-9. doi:10.1016/0031–9422(90)83059–A. http://dx.doi.org/10.1016/0031-9422(90)83059-A
28. Ishii H Alkaloids of rutaceous plants XVI, Alkaloids of Zanthoxylum schinifolium. J Pharm Soc Jap. 1961;81:1633-5.
29. Hufford C. Aporphine Alkaloids of Zanthoxylum simulans and Z. nigrescen. Phytochemistry. 1976;15:1169. http://dx.doi.org/10.1016/0031-9422(76)85123-0
30. Chen IS, Wu SJ, Leu YL, Tsai IW, Wu TS. Alkaloids from root bark of Zanthoxylumsimulans. Phytochemistry. 1996;42(1):217-9. doi:10.1016/0031–9422(95)00856–X. http://dx.doi.org/10.1016/0031-9422(95)00856-X
31. Kashiwanda Y,Chikashi I, Hitoshi K, Izumi M, Katsuko K, Suneo N, *et al*. Alkylamides of the Fruit of Zanthoxylum SSP. Phytochemistry. 1997;44(6):1125–7. http://dx.doi.org/10.1016/S0031-9422(96)00683-8
32. Xiong Q, Dawen S, Yamamoto H, Mizuno M. Alkylamides from pericarps of Zanthoxylum bungeanum. Phytochemistry. 1997;46(6):1123-6. doi:10,1016/S0031–9422(97)84398–1.
33. Sheng C, Tzu C, Ya C, Che T, Wei–Shan Z. Chemical Constituents and Biological Activity of the Fruit of Zanthoxylum integrifoliolum. J Nat Prod. 1999;62:833-7. http://dx.doi.org/10.1021/np980471n PMid:10395498
34. Cheng M–J, Lee K–H, Tsai I–L, Chen I–S. Two new sesquiterpenoids and anti–HIV principles from the root bark of Zanthoxylum ailanthoides. Bioorgan Med Chem. 2005;13(21):5915-20,doi:10.1016/j.bmc.2005.07.050. http://dx.doi.org/10.1016/j.bmc.2005.07.050
35. Ross SA, Al–Azeib MA, Krishnaveni KS, Fronczek FR, Burandt CL. Alkamides from the leaves of Zanthoxylumsyncarpum. J Nat Prod 2005;68:1297-9. http://dx.doi.org/10.1021/np0580558 ; PMid:16124784
36. Boulware RT, Stermitz FR. Some alkaloids and other constituents of Zanthoxylummicrocarpum and Z. procerum. J Nat Prod. 1981;44(2):200-5. http://dx.doi.org/10.1021/np50014a010
37. Mu-oz MA, Torres R, Cassels BK. Aurapten and Flindersine From Zanthoxylum coco. J Nat Prod. 1982;45(3):367-9.doi:10.1021/np50021a023. http://dx.doi.org/10.1021/np50021a023
38. Chang C–T, Doong S–L, Tsai I–L, Chen I–S. Coumarins and anti–HBV constituents from Zanthoxylum schinifolium. Phytochemistry. 1997;45(7):1419-22. doi:10,1016/S0031–9422(97)89023–1.
39. Cuca SLE, Martinez VJC, Monache FD. 79´–Epoxylignan and other constituents of Zanthoxylum culantrillo. Phytochemistry. 1998;47(7):1437-9. doi:10.1016/S0031–9422(97)00747–4. http://dx.doi.org/10.1016/S0031-9422(97)00747-4
40. Yang Y–P, Cheng M–J, Teng C–M, Chang Y–L, Tsai I–L, Chen I–S. Chemical and anti–platelet constituents from Formosan Zanthoxylum simulans, Phytochemistry. 2002;61(5):567-72. http://dx.doi.org/10.1016/S0031-9422(02)00268-6
41. Mukhlesur RM, Anwarul IM, Khondkar P, Gray AI. Alkaloids and lignans from Zanthoxylum budrunga (Rutaceae). Biochem. Syst Ecol. 2005;33(1):91-6. doi:10.1016/j.bse.2004.04.016. http://dx.doi.org/10.1016/j.bse.2004.04.016
42. Zhou X–J, Chen X–L, Li X–S, Su J, He J–B, Wang Y–H, *et al*. Two dimeric lignans with an unusual αβ–unsaturated ketone motif from Zanthoxylum podocarpum and their inhibitory effects on nitric oxide production.Bioorgan. Med Chem Letters. 2011;21(1):373-6. doi:10,1016/j,bmcl,2010,10,135.
43. Niu X–F, Zhou P, Li W–F, Xu H–B. Effects of chelerythrine a specific inhibitor of cyclooxygenase–2 on acute inflammation in mice. Fitoterapia. 2011;82(4):620-5. doi:10.1016/j.fitote.2011.01.020. http://dx.doi.org/10.1016/j.fitote.2011.01.020
44. Guo T, Deng Y–X, Xie H, Yao C–Y, Cai C–C, Pan S, *et al*. Antinociceptive and anti–inflammatory activities of ethyl acetate fraction from Zanthoxylum armatum in mice. Fitoterapia. 2011;82(3):347-51. doi:10.1016/j.fitote.2010.11.004. http://dx.doi.org/10.1016/j.fitote.2010.11.004
45. Xiong Q, Dawen S, Mizuno M. Flavanol glucosides in Pericarps of Zanthoxylum bungeanum. Phytochemistry.1994;39:723-9. http://dx.doi.org/10.1016/0031-9422(94)00965-V
46. Sati SC, Sati MD, Raturi R, Badoni PP, Singh H. A New Flavonoidal Glycoside From stem bark of Zanthoxylum armatum. International Journal of Pharmaceutical Innovations (IJPI's). J Pharm Herb Form. 2011;1(2):29-32.
47. Cho JY, Hwang T, Chang TH, Lim Y, Sung P, Lee T, *et al*. New coumarins and anti–inflammatory constituents from Zanthoxylum avicennae. Food Chem. 2012;135:17-23. http://dx.doi.org/10.1016/j.foodchem.2012.04.025
48. Waterman PG, Grundon MF. Chemistry and chemical taxonomy of the Rutales, Academic Press,1983.
49. Waterman PG. Chemosystematics of the Rutaceae: Comments on the interpretation of da Silva & al. Plant Syst. Evol. 1990;173(1-2):39-48. doi:10,1007/BF00937761.
50. Thuy TT, Porzel A, Ripperger H, Sung TV, Adam G. Bishordeninyl terpene alkaloids from Zanthoxylum avicennae. Phytochemistry. 1999;50(5):903-7. doi:10,1016/S0031–9422(98)00612-8.
51. Adesina SK. The Nigerian Zanthoxylum; Chemical and Biological Values. Afr J Tradit Complem. 2005;3:282–301. http://dx.doi.org/10.4314/ajtcam.v2i3.31128
52. Pati-o LOJ, Prieto RJA, Cuca SLE. Zanthoxylum genus as potential source of bioactive compounds. Bioactive Compounds in Phytomedicine. 2008;185–218.
53. Fotie J, Bohle DS, Leimanis ML, Georges E, Rukunga G, Nkengfack AE. Lupeol Long-Chain Fatty Acid Esters with Antimalarial Activity from Holarrhena floribunda. J Nat Prod. 2006;69(1):62-7. http://dx.doi.org/10.1021/np050315y; PMid:16441070
54. Kamikado T, Chang CF, Murakoshi S, Sakurai A, Tamura S. Isolation and structure elucidation of growth inhibitors on silkworm larvae from Magnolia kobus DC. Agr Biol Chem.Tokyo.1975;39(4):833-6. http://dx.doi.org/10.1080/00021369.1975.10861682 ; http://dx.doi.org/10.1271/bbb1961.39.833
55. Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrob. Agent CH. 2004;48(5):1803-6. http://dx.doi.org/10.1128/AAC.48.5.1803-1806.2004 PMCid:PMC400546
56. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193:673-5. http://dx.doi.org/10.1126/science.781840 ; PMid:781840
57. Johnson JA, Dennull RA, Gerena L, Lopez-Sanchez M, Roncal NE, Waters NC. Assessment and continued validation of the malaria SYBR Green I-based fluorescence assay for use in malaria drug screening. Antimicrob. Agent CH. 2007;51(6):1926-33. http://dx.doi.org/10.1128/AAC.01607-06 ; PMid:17371812 PMCid:PMC1891422
58. Akala HM, Eyase FL, Cheruiyot AC, Omondi AA, Ogutu BR, Waters NC, *et al*. Antimalarial drug sensitivity profile of Western Kenya Plasmodium falciparum field isolates determined by a SYBR Green I in vitro assay and molecular analysis. Am J Trop Med Hyg. 2011;85:34-41. http://dx.doi.org/10.4269/ajtmh.2011.10-0674; PMid:21734121 PMCid:PMC3122340
59. Adesina KS, Johannes R. Arnottianamide and other constituents of Zanthoxylum gilletii Root. J Nat Prod. 1988;5:60-602,
60. Ng K, Gray AI, Waterman PG. Benzophenanthridine alkaloids from the stem bark of a Zanthoxylum species. Phytochemistry. 1987;26(12):3251-4. http://dx.doi.org/10.1016/S0031-9422(00)82481-4
61. Martinez–Martinez FJ, Padilla–Martinez II, Hernandez–Carlos B, Perez–Gutierrez RM, Garcia–Baez EV. X–ray diffraction and total 1H and 13C NMR assignment of (RS)–5 6–dihydro–78–dimethoxy–5–methyl–6–(2–oxopropyl)–(23–methylenedioxyphenyl)–[c]–phenanthridine ((RS)–6–acetonyldihydrochelerythrine). J Chem Crystallogr. 2002;32(3–4):63–68. http://dx.doi.org/10.1023/A:1015656625468
62. Chang YC, Hsieh PW, Chang FR, Wu RR, Liaw CC, Lee KH, *et al*. Two new protopinesargemexicaines A and B and the anti–HIV alkaloid 6–acetonyldihydrochelerythrine from Formosan Argemone mexicana. Planta Med. 2003;69(2):148-52. http://dx.doi.org/10.1055/s-2003-37710; PMid:12624820
63. Cai M, Zhou Y, Wang X, Li R, Liao X, Ding L. Rapid structural characterization of isomeric benzo[c]phenanthridine alkaloids from the roots of Zanthoxylum nitidium by liquid chromatography combined with electrospray ionization tandem mass spectrometry. Rapid Commun. Mass. SP 2007;21(12):1931-6. http://dx.doi.org/10.1002/rcm.3045; PMid:17510939
64. Gaya CH, Kawaka JF, Muchugi A, Ngeranwa JJ, Variation of alkaloids in the Kenyan Zanthoxylum gilletii (De Wild Waterman). Afr J Plant Sci. 2013;7(9):438-44. http://dx.doi.org/10.5897/AJPS2013.1008

65. O'Connell MM, Bentley MD, Campbell CS, Cole BJ. Betulin and lupeol in bark from four white–barked birches. Phytochemistry. 1988;27(7):2175-6. http://dx.doi.org/10.1016/0031-9422(88)80120-1
66. You YJ, Nam NH, Kim Y, Bae KH, Ahn BZ. Antiangiogenic activity of lupeol from Bombax ceiba, Phytother. Res. 2003;17(4):341-4. http://dx.doi.org/10.1002/ptr.1140 PMid:12722136
67. Agarwal RB, Rangari VD. Antiinflammatory and antiarthritic activities of lupeol and 19 alpha–H lupeol isolated from Strobilanthus callosus and Strobilanthus ixiocephala roots. Indian J Pharmacol. 2003;35(6):384-7.
68. Batista R, Júnior ADJS, De Oliveira A. Plant–derived antimalarial agents: new leads and efficient phytomedicines, Part II, Non–alkaloidal natural products. Molecules. 2009;14:3037-72 http://dx.doi.org/10.3390/molecules14083037; PMid:19701144
69. Basco LK, Mitaku S, Skaltsounis AL, Ravelomanantsoa N, Tillequin F, Koch M, *et al*. In vitro activities of furoquinoline and acridone alkaloids against Plasmodium falciparum. Antimicrob. Agent CH. 1994;38(5):1169-71. http://dx.doi.org/10.1128/AAC.38.5.1169
70. Ward RS. The synthesis of lignans and neolignans. Chem Soc Rev. 1982;11(2):75-125. http://dx.doi.org/10.1039/cs9821100075
71. Kucukboyaci N, Sener B. Biological activities of lignans from Taxus baccata L, growing in Turkey. J Med Plants Res. 2010;4(12):1136-40.
72. Wagner H Plant phenolic compounds in plants of pharmaceutical interest, In: T, Swain http://dx.doi.org/10.1007/978-1-4684-3372-2_18
73. Kupeli E, Erdemoglu N, Yesilada E, Sener B. Anti–inflammatory and antinociceptive activities of taxoids and lignans from the heartwood of Taxus baccata L. J Ethnoparmacol. 2003;89:123-9. http://dx.doi.org/10.1016/j.jep.2003.09.005
74. MacRae WD, Towers GN. Biological activities of lignans. Phytochemistry. 1984;23(6):1207-20. http://dx.doi.org/10.1016/S0031-9422(00)80428-8
75. Erdemoglu N, Sener B, Choudhary MI. Bioactivity of lignans from Taxus baccata. Z. Naturforsch C. 2004;59(7–8): 494–498. PMid:15813367
76. Ortet R, Prado S, Regalado EL,Valeriote FA, Media J, Mendiola J, *et al*. Furfuran lignans and a flavone from Artemisia gorgonum Webb and their in vitro activity against Plasmodium falciparum. J Ethnopharmacol. 2011;138(2):637-40. http://dx.doi.org/10.1016/j.jep.2011.09.039 ; PMid:21982788
77. de Andrade–Neto VF, da Silva T, Lopes LMX, do Rosário VE, de PillaVarotti F, Krettli AU. Antiplasmodial activity of aryltetralonelignans from Holostylis reniformis. Antimicrob Agent CH. 2007;51(7):2346-50. http://dx.doi.org/10.1128/AAC.01344-06 ; PMid:17438049 PMCid:PMC1913279
78. Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Christensen SB, Sittie A, *et al*. New anti–HIV–1 antimalarial and antifungal compounds from Terminalia bellerica. J Nat Prod. 1997;60(7:739-42.
79. Kraft C, Jenett-Siems K, Köhler I, Tofern-Reblin B, Siems K, Bienzle U, *et al*. Antiplasmodial activity of sesquilignans and sesquineolignans from Bonamia spectabilis. Phytochemistry. 2002;60(2):167-73. http://dx.doi.org/10.1016/S0031-9422(02)00101-2
80. Ma C, Zhang HJ, Tan GT, Hung NV, Cuong NM, Soejarto DD, *et al*. Antimalarial Compounds from Grewia bilamellata. J Nat Prod. 2006;69(3):346-50. http://dx.doi.org/10.1021/np050313d ; PMid:16562832
81. da Silva Filho AA, Costa ES, Cunha WR, Silva ML, Nanayakkara NP, Bastos JK. In vitro antileishmanial and antimalarial activities of tetrahydrofuran lignans isolated from Nectandra megapotamica (Lauraceae). Phytother Res. 2008;22(10):1307-10. http://dx.doi.org/10.1002/ptr.2486 ; PMid:18688887
82. Abrantes M, Mil–Homens T, Duarte N, Lopes D, Cravo P, Madureira MD, *et al*. Antiplasmodial activity of lignans and extracts from Pycnanthus angolensis. Planta Med. 2008;74(11):1408-12. http://dx.doi.org/10.1055/s-2008-1081317; PMid:18671202
83. Rodríguez–Guzmán R, Fulks L, Radwan MM, Burandt CL, Ross SA. Chemical constituents antimicrobial and antimalarial activities of Zanthoxylum monophyllum. Planta Med. 2011;77(13):1542-4. http://dx.doi.org/10.1055/s-0030-1270782; PMid:21341176


## PICTORIAL ABSTRACT




## SUMMARY


- *The stem bark of Zanthoxylum gilletii showed good antiplasmodial activities with $IC_{50}$ values < 2.52 µM*
- *Sesamin and 8-acetonyldihydrochelerythrine showed the highest antiplasmodial activities with $IC_{50}$ values < 4.06 µM*
- *Six compounds were isolated from the stem bark of Z. gilletii*
- *Sesamin, 8–acetonyldihydrochelerythrine and lupeol are reported here for the first time from this species*


## ABOUT AUTHORS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