

Anti-Toxoplasma gondii screening of eight species used in Côte d'Ivoire, and bio-guided isolation of three previously undescribed nor-sesquiterpenoids and one benzyl glycoside from Elaeis guineensis leaves

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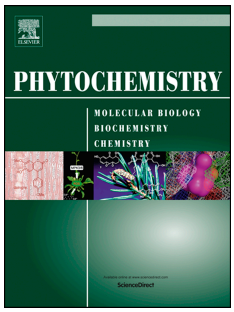
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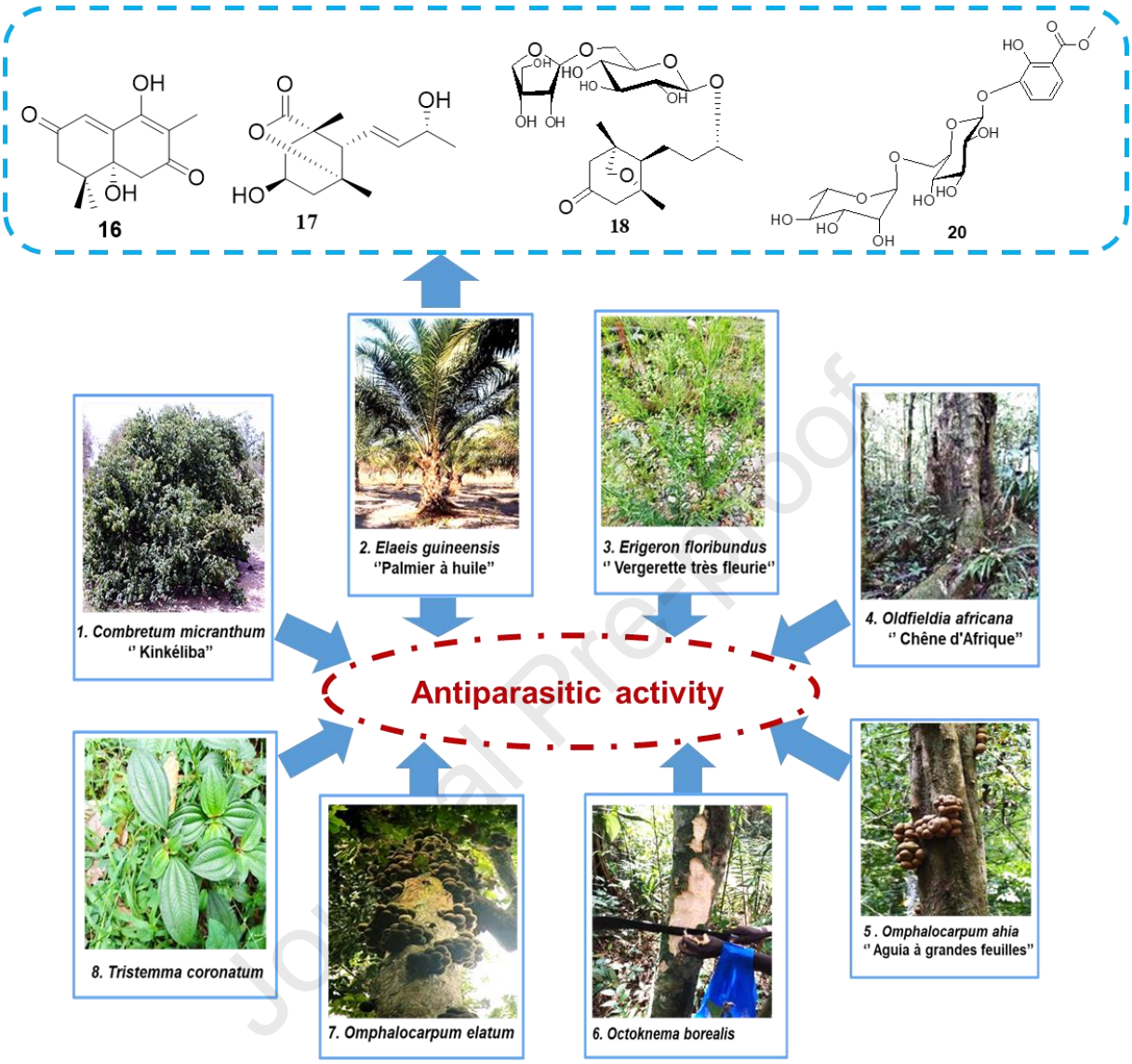
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REVISED GRAPHICAL ABSTRACT



Images (1-8) represent the eight plant drugs collected and tested against *T. gondii*. MeOH 80% extract of *E. guineensis* leaves was proven to be bioactive and allowed the isolation of previously undescribed compounds **16-18** and **20**.

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Nangouban Ouattara[a,b,d*], Mansour Issoufou-Tiny[a], Abdulmagid Alabdul Magid[a], Sandie Escotte-Binet[b,c], Philomène Akoua Yao-Kouassi[d,e], Isabelle Villena[b,c], Laurence Voutquenne-Nazabadioko[a].

[a] Université de Reims Champagne-Ardenne, CNRS, ICMR 7312, 51097, Reims, France.
[b] Université de Reims Champagne Ardenne, ESCAPE UR 7510, 51097, Reims, France.
[c] Centre National de Référence de la Toxoplasmose, CHU Reims, 51092, Reims, France.
[d] Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et de Technologie, Université Félix HOUPHOUET-BOIGNY 22 BP 582 Abidjan, Côte d'Ivoire.
[e] Université Polytechnique de San Pedro, BP V1800 San Pedro, Côte d'Ivoire.

*Corresponding authors
E-mail address: nangouban.ouattara@u-bordeaux.fr (Dr Nangouban OUATTARA)

Abstract

The apicomplexan parasite *Toxoplasma gondii* causes toxoplasmosis, a ubiquitous and cosmopolitan parasitosis, generally asymptomatic and potentially dangerous for the fetus and highly immunocompromised patients. Pyrimethamine and sulfadiazine, supplemented with folic acid, are the drugs of choice to treating the disease, but they produce severe side effects and treatments fail due to drug resistance. New anti-*Toxoplasma* compounds are needed, and natural compounds can be a good source for obtaining them. The antiparasitic activity of 40 polar and non-polar extracts of eight antiparasitic medicinal plants used in Côte d'Ivoire, and selected based on ethnopharmacological survey, were evaluated *in vitro* against *T. gondii*. Among them, the hydromethanolic extract of the *Elaeis guineensis* leaves exhibited the best parasite growth inhibition (94% ± 0.07) at 25 µg/mL without being cytotoxic at the same dose. The fractionation of this extract did not allow the recovery of antitoxoplasmic activity in its individualized fractions. The ^{13}C-NMR based dereplication of this extract and the purification of two fractions highlighted the presence of saccharides (**1-2**), aliphatic acids (**3** and **8-11**), cardanols (**12-13**), triterpene (**14**), sterol (**15**), nor-sesquiterpene (**16**), megastigman derivatives (**7** and **17-19**), glycosylated benzyl (**20-21**), flavonoids (**4-6** and **22**), pseudoalkaloid (**23**), and allyl glycoside (**24**). Nine compounds **1-9** were identified during the dereplication process, whereas fifteen compounds **10-24** were identified after purification and four of them were undescribed (**16-18** and **20**). A synergistic effect between these metabolites is thought to be responsible for the anti-*Toxoplasma* activity. However, further studies should be carried out to confirm and explain this synergistic or antagonistic effect between the identified compounds.

Keywords: *Toxoplasma gondii,* Vero cell, cytotoxicity, bioguided fractionation, NMR, dereplication, medicinal plants*, Elaeis guineensis,* nor-sesquiterpene, megastigman derivatives, benzyl glycoside

1. Introduction

Elaeis guineensis Jacq. (Arecaceae) is-the West African oil palm tree, reaching 20-25 m height, with a stipe measuring between 22-75 cm in diameter. Its leaves are pinnate and the petiole is robust and thorny. Flavonoids, tannins, saponins, phenolic compounds, alkaloids, and tocotrienols were previously identified in the leaves (Che Zain et al., 2021; Yin et al., 2013; Horvath et al., 2006). Infusion of the leaves, either alone or in alcohol, is employed as an anti-parasitic remedy, particularly against malaria in Côte d'Ivoire. Nevertheless, oxidised palm oil has been demonstrated to cause kidney, lung, liver and heart toxicity (Ebong et al., 1999). The hydromethanolic extracts of the leaves exhibit antimalarial activity against *P. falciparum* (Annan et al., 2012), and antimicrobial activities (Ebong et al., 1999; Yin et al., 2013).

Toxoplasmosis is a ubiquitous disease of parasitic origin caused by an obligate intracellular eukaryote *Toxoplasma gondii,* whose final host is a felid (Frenkel, 1974). Although most human infections are uncomplicated, the parasite can persist and the infection can be reactivated in immunosuppressed patients leading to cerebral or generalized toxoplasmosis in immunocompromised patients (Robert-Gangneux et al., 2022). In add, when infection is acquired during pregnancy, it can cause congenital toxoplasmosis with serious consequences for the fetus (Wong and Remington, 1994; McLeod et al., 2009; Codiacconi et al., 2020) or the infant. *T. gondii* could also cause severe ocular disease in immunocompetent patients (Holland, 2004; Kim et al., 2013) and in congenital toxoplasmosis (Bosch-Driessen et al., 2002). It is estimated in a recent meta-analysis that about 30-35% of the world's population is infected with *T. gondii* (Bigna et al., 2020). Toxoplasmosis is ranked as the third largest contributor to the health burden caused by foodborne illness in Europe (De Sousa et al., 2023). In Côte d'Ivoire, the latest studies Epidemiological studies have shown that seroprevalence has decreased from 80% in the 1960s to 45 % in 2014 (Bonouman-Ira et al., 2017). The current treatment of acute toxoplasmosis is the combination of sulfadiazine and pyrimethamine, which act synergistically to block the folate pathway, involved in DNA synthesis with the dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS) enzymes. Unfortunately, this inhibition also creates a deficiency in. the host leading to adverse effects (hematopoiesis and foetological disorders) (Dujovne et al., 1967).

In addition to these side effects, there is drug resistance (Anacleto-Santos et al., 2023); therapeutic failures (Derouin, 2001; Baatz et al., 2006), and lack of a human vaccine shows that it is of great importance to identify novel potent candidates that would be well-tolerated and act on both tachyzoites and cysts (Mamaghani et al., 2023). The antiparasitic properties of natural products, such as plant derived substances, have been extensively investigated (Sepulveda-Arias et al., 2014). A review carried out between the period 1981-2019 stated that out of 20 antiparasitic drugs proven as a treatment, there were, nine derived from natural products and three were derived from synthetic pharmacophoric sources of natural products (Newman and Cragg, 2020). In recent years, several studies have been conducted *in vitro* and or *in vivo* that have shown that plant species could effectively inhibit the growth of *T. gondii* (Anacleto-Santos et al., 2023; Darme et al., 2022; Darme et al. 2021).

In this study, eight plant species were selected, based on a literature survey on plants most widely used by traditional healers in Côte d'Ivoire to treat bacterial and parasitic diseases, and for which anti-*Toxoplasma* activity had never been evaluated. These included: *Combretum micranthum* G. Don (Combretaceae) leaves, *Elaeis guineensis* Jacq. (Arecaceae) leaves, *Erigeron floribundus* Kunth Sch.Bip (Asteraceae) aerial parts, *Oldfieldia africana* Benth. & Hook. F. (Picrodendraceae) stem bark, *Octoknema borealis* Hutch. & Dalziel (Olacaceae) leaves and stem bark, *Omphalocarpum ahia* A. Chev. (Sapotaceae) leaves and stem bark, *Omphalocarpum elatum* Miers stem bark, and whole plant of *Tristemma coronatum* Benth (Melastomataceae). *C. micranthum*, commonly called Kinkeliba, is used in West Africa as a traditional medication for the treatment of malaria, diabetes, high blood pressure and inflammatory diseases. Its leaves extracts showed antitoxoplasmosis (Sepulveda-Arias et al., 2014), antibacterial (Banfi et al., 2014), antiviral activity (Ferrea et al., 1993), and protective effect against kidney damage (Osonwa et al., 2012). *E. floribundus*, a terrestrial herbaceous plant, is used in Côte d'Ivoire for the treatment of skin disorders, dyspepsia, abdominal pains, various diseases of microbial origin (Koné et al., 2002), in AIDS therapy, rheumatism, gout, cystitis, nephritis, dysmenorrhoea, dental pain, headache (Valnet, 1983; Burkill, 1985). The aerial parts extracts showed antimicrobial (Atindenou et al., 2002), antifungal (Tra Bi et al., 2008), anthelminthic (Diehl et al., 2004), anti-*Trypanosoma brucei* (Petrelli et al., 2016), and antimalarial effect (Tepongning et al., 2018). *O. africana* is a large tree considered in Côte d'Ivoire as a powerful fetish tree with efficacious medicinal virtues. The bark is antiseptic and haemostatic. A decoction of the bark is added to baths and draughts to treat blennorrhoea and act as a pelvic decongestant (Burkill, 1985). The stem bark extracts showed antimalarial (Rusaati et al., 2012), antibacterial and antifungic activities (Berinyuy, 2014). The stem bark of *O. borealis*, a large tree, are used in traditional medicine for the treatment of pulmonary troubles whereas the leaves are used as febrifuges (Abbiw, 1990; Burkill, 1985). *O. ahia* is an evergreen medium-sized tree. It is used in folklore medicine for the treatment of pain, inflammation, bacterial and parasitic diseases (Armah et al., 2016; Burkill, 1997). The stem bark extracts showed antibacterial, anti-leishmania and anti-inflammatory activities (Alake et al., 2021). *O. elatum* is a large tree from West African, used traditionally for the treatment of sterility of males and increases lactation in women. A decoction of the bark, combined with the fruits of peppers (*Capsicum annuum*) and *Solanum anguivi*, is used to treat malaria. A decoction of the young leaves is used for the treatment of cough. Its stem bark extracts showed high *in vitro* anthelmintic (Nyasangox et al., 2018), anti-inflammatory and antimicrobial activities (Sandjo et al., 2014). *T. coronatum* is a semi-prostrate herb or shrub to nearly 1 m high in the ground vegetation of the closed forest, from Guinea to Togo. Leaves are used in folklore medicine as pain-killers, sedatives, for arthritis and rheumatism (Burkill, 1985), against typhoid fever, hemorrhoids, infertility, and skin diseases (Nguenang et al., 2018). Its leaves extracts showed antibacterial activity against a strain of *Pseudomonas aeruginosa* and strains of *Staphylococcus aureus*, as well as antifungal activity against *Candida* species, such as *C. albicans*, *C. tropicalis* and *C. glabrata* (Ahoua et al., 2015).

In this study, *n*-heptane (*n*-Hept), ethyl acetate (EtOAc), dichloromethane (DCM) and aqueous methanol 80% (MeOH 80%) extracts were prepared from the ten selected plants and tested *in vitro* at 25 µg/mL to evaluate their activity against *T. gondii*, and cytotoxicity on normal Vero cell line. In addition, a ^{13}C-NMR-based dereplication workflow combined with a bioactivity-guided fractionation process was applied on the most active extract (MeOH 80% extract of the leaves of *E. guineensis*) to identify the compounds being involved in the biological effect against *T. gondii*. This paper describes the isolation and characterization of three nor-sesquiterpenes and one benzyl glycoside previously undescribed.

2. Results and Discussions

2.1. Identification of compounds from Elaeis guineensis

Chemical profiling of the six fractions HP_{1A}–HP_5 by ^{13}C-NMR based dereplication was initiated in order to identify the main chemicals that could potentially be involved in the activity of the MeOH 80% extract of *E. guineensis* leaves (Fig. 1). Nine compounds, including two saccharides (**1**-**2**), three flavonoids (**4**-**6**), one megastigman derivative (**7**), three aliphatic acid derivatives (**3** and **8**-**9**) were identified. The correlated chemical shifts of cluster 1 in fraction HP_{1A} were assigned to glucopyranose (**1**) and *α*-galactopyranosyl-(1→3)-*β*-galactopyranoside (**2**) (Agrawal, 1992). Similarly, cluster 2 in fraction HP_{1B} proposed 1-monopalmitin (**3**) (Hu et al., 2022). The cluster 3 in fraction HP_3 proposed vicenin-2 (**4**) (Islam et al., 2014) and vitexin-2"-*O*-*β*-D-glucopyranoside (**5**) (Pauli and Junior, 1995). The cluster 4 in fraction HP_3 proposed vitexin (**6**) (Kim et al., 2005). The cluster 5 in fraction HP_4 proposed roseoside (**7**) (Matsunami et al., 2010) whereas cluster 6 in fraction HP_5 proposed elaidic acid methyl ester (**8**) and linoelaidic acid methyl ester (**9**) (Alexandri et al., 2017) (Figure 1). The identification of these compounds was confirmed by comparison with literature data and by analysis of HR-ESI-MS spectra of HP_{1A}–HP_5.

The HP_3 fraction was purified giving four flavonoids (**4**-**6** and **22**), four megastigman derivatives (**7** and **17**-**19**), two benzyl glycosides (**20**-**21**), one pseudo alkaloid (**23**) and one allyl glycoside (**24**). Their structures were elucidated through the use of 1D and 2D-NMR experiments, in conjunction with the analysis of HR-ESI-MS data and by comparison with previously reported values. Three compounds **17**-**18** and **20** were undescribed and the know compounds are vicenin-2 (**4**) (Islam et al., 2014), vitexin-2''-*O*-glucoside (**5**) (Pauli & Junior, 1995), vitexin (**6**) (Kim et al., 2005), scutellarein-8-*C*-*β*-D-glucoside (**22**) (Kim et al., 2015), roseoside (**7**) (Matsunami et al., 2010), byzantionoside B (**19**) (Matsunami et al., 2010), hydrangeifolin I (**21**) (Hamerski et al., 2005), 2-ethyl-3-methylmaleimide N-*β*-D-glucopyranoside (**23**) (Krajewski et al., 1996) and erigeside F (**24**) (Yamamura et al., 1998) (Figure 2). Likewise, HP_5 fraction was purified giving four aliphatic acid derivatives (**8**-**11**), two cardanol derivatives (**12**-1**3**), one triterpene (**14**), one sterol (**15**) and one undescribed nor-sesquiterpene (**16**). The known compounds are elaidic acid methyl ester (**8**), linoelaidic acid methyl ester (**9**), palmitic acid (**10**), palmitic acid methyl ester (**11**) (Alexandri et al., 2017), 3-[12(*E*)-pentadecenyl]phenol (**12**), 3-[14(*E*)-

heptadecenyl]phenol (**13**) (Liu and Abreu, 2006), ursolic acid (**14**) (Mahato and Kundu, 1994), and stigmasta-4,24(28)-dien-3-one (**15**) (Sheu et al., 1999) (Figure 2).

Compound **16** has the molecular formula $C_{13}H_{16}O_4$, deduced from the protonated molecular ion at *m/z* 237.1125 $[M + H]^+$ (calcd for $C_{13}H_{17}O_4$, 237.1127), in positive mode HR-ESI-MS. The ^1H NMR and ^1H–^1H COSY spectra displayed signals of three methyl groups at δ_H 1.25, 1.26 and 1.74, two methylene protons at δ_H 2.44 (1H, d, J = 17.2 Hz), 2.55 (1H, d, J = 17.2 Hz), 2.67 (1H, d, J = 18.4 Hz), 3.07 (1H, d, J = 18.4 Hz) and one ethylenic proton at δ_H 6.95 (1H, br. s) (Table 1). This, in a combination with signals in ^{13}C NMR and HSQC spectra of two ketones at δ_C 195.7 and 201.0, four ethylenic carbons at δ_C 103.1, 131.9, 142.8 and 160.0, two methylene carbons at δ_C 39.9 and 50.5, two quaternary carbons at δ_C 39.0 and 83.8, and three methyl carbons at δ_C 17.5, 21.9 and 23.2, suggest a nor-sesquiterpene derivative (ITO et al., 1997) (Fig. 2). The ketone at δ_C 201.0 (C-3) correlates in HMBC with the methylene protons $H_{\alpha\beta}$-2 at δ_H 2.67 and 3.07, coupled together with a geminal coupling constant of J = 18.4 Hz, which indicates that these protons were between two quaternary carbons. HMBC correlations were observed from $H_{2\alpha\beta}$-2 to carbons at δ_C 39.0 (C-1), 83.8 (C-6) and 131.9 (C-4), from C-1 to the methylene protons $H_{\alpha\beta}$-7 at δ_H 2.44 (d, J = 17.2 Hz) and 2.55 (d, J = 17.2 Hz), and the methyl protons H-13 (δ_H 1.25) and H-12 (δ_H 1.26), and from $H_{\alpha\beta}$-7 to the second ketone at δ_C 195.7 (C-8) (Fig. 3). Similarly, HMBC correlations were observed between methyl protons H-13 and carbons C-12 and C-6. The ethylenic proton H-4 at δ_{H1} 6.95 (δ_{C4} 131.9) showed HMBC correlations with two ethylenic carbons at δ_C 142.8 (C-5) and 160.0 (C-11), attached to a hydroxyl. Finally, the methyl protons at δ_H 1.74 (H-10), were correlated in the HMBC with the quaternary ethylenic carbon C-9 at δ_C 103.1 (Fig. 3). The absolute configurations of C-6 was established (6S) by comparing the experimental ECD spectra of **16** with the published (-)-(6S)- eucomegastigmane A (Yan et al., 2017). The $^{20}[\alpha]_D$ -0.3° (*c* 0.1; MeOH) confirm the stéréochemistry. Thus, the structure of compound **16** is (-)-(6S)-4,5,6,7-tetrahydro-5,9-dihydroxy-4,4,8-trimethyl-2(3*H*),7-naphthalendione which named (-)-(6S)-elaeisdione.

Compound **17** has the molecular formula $C_{13}H_{20}O_4$, deduced from the protonated molecular ion having lost one or two water molecules at *m/z* 223.1333 $[M - H_2O + H]^+$ (calcd $C_{13}H_{19}O_3$, 223.1312) or 205.1223 $[M - 2H_2O + H]^+$ (calcd $C_{13}H_{17}O_2$ 205.1226) (Hu and al., 2022), in positive mode HR-ESI-MS. Analysis of the ^1H NMR, ^{13}C NMR, COSY, ROESY, HSQC and HMBC spectra led to the identification of a cucubalactone derivative (Cheng et al., 2002) (Figs. 2 and 4), a nor-sesquiterpene megastigman. The ^{13}C-NMR spectrum displayed thirteen signals: three methyls (δ_C 17.8, 22.2, 22.3), two methylenes (δ_C 35.8, 37.5), three methynes (δ_C 57.5, 64.5, 67.3), two ethylenic carbons (δ_C 120.3, 142.4), two quaternary signals (δ_C 46.2, 85.5) and one carbonyl at δ_C 180.0. The signals at δ_C 64.5, 67.3, and 85.5 were characterized as carbons bearing an oxygen atom. The similarity of the NMR data of **17** and cucubalactone showed that they had an identical carbon skeleton (Table 1). The main difference in the ^{13}C-NMR spectra was the presence of one supplementary hydroxy substituent at C-6 in cucubalactone (Cheng et al., 2002). The specific chemical shift of the tertiary carbon C-3 at 64.5 ppm (δ_{H3} 3.80) is indicative of the presence of a tertiary alcohol in a ring. This correlated in HMBC with the methylene

protons $H_{\alpha\beta}$-2 at δ_H 1.62 (dt, J = 13.7, 6.5 Hz) and 1.76 (dd, J = 13.7, 7.3 Hz) and $H_{\alpha\beta}$-4 at δ_{H4} 1.64 (dd, J = 13.8, 6.8 Hz), and 2.11 (dd, J = 13.8, 6.8 Hz), allowing their respective positioning. Similarly, the protons $H_{\alpha\beta}$-4 correlated in HMBC with the methyl C-13 (δ_C 22.2), carbons C-2 (δ_C 35.8), C-6 (δ_C 57.5), C-3 (δ_C 64.5) and C-5 (δ_C 85.5). The protons $H_{2\alpha\beta}$-2 showed HMBC correlation with the methyl C-11 (δ_C 17.8), carbons C-4 (δ_C 37.5), C-1 (δ_C 46.2), C-3, C-6 and the carbonyl C-12 at δ_C 180.0 ppm (Fig. 4). The lactone function between C-12 and the quaternary carbon C-5 (δ_C 85.5) was corroborated by their specific chemical shifts (Cheng et al., 2002). HMBC correlations from C-5 to protons H-6, H_2-4, H-13 and H-7 suggested the position of the lateral chain. The ethylenic proton H-7 at δ_H 5.77 (1H, dd, J = 15.4, 9.6 Hz) is coupled in COSY spectrum with the vicinal protons H-6 (δ_H 2.49, d, J = 9.6 Hz) and H-8 (δ_H 5.87, dd, J = 15.4, 5.4 Hz), which correlated in COSY with it vicinal proton H-9 at δ_H 4.31 (1H, q, J = 6.4 Hz/δ_C 67.3). The coupling constant J = 15.4 Hz indicated that this ethylenic group was in the *trans* configuration (Fig. 4). Carbon C-9 correlated in HMBC with the methyl protons H-10 at δ_H 1.28 (3H, d, J = 6.2 Hz/δ_{C10} 22.3). Finally, the stereochemistry of carbons C-3, C-6 and C-9 was established by the ROESY correlations between H-3 with H_{eq}-4, H-6 with H-11, H-6 with H-13 and H-7 with H-9 or H-8 with H-9, showing that they belong to the same chemical environment, respectively. Thus, the structure of compound **17** was elucidated to be 1*β*,5*β*-dimethyl-3*β*-hydroxy-6*α*-(3-hydroxy-1-butenyl)-12-oxobicyclo-[3.2.1]-13-oxaoctane, which was named 6-dehydroxy-cucubalactone or elaeisdiol.

Compound **18** has the molecular formula $C_{24}H_{40}O_{12}$ deduced from the sodium ion at *m/z* 543.2418 $[M + Na]^+$ (calcd $C_{24}H_{40}O_{12}Na$, 543.2417), in positive mode HR-ESI-MS. The ^1H NMR and $^{13}C_{J\text{-modulated}}$ NMR spectral data of compound **18** showed similarity to that of compound **17** (Table 1). The difference was presence of a ketone at C-3 (δ_C 211.2) rather than a hydroxyl, an oxygen-linked methylene forming a cyclised ether-oxy function at C-12 (δ_H 3.58/δ_C 78.0) rather than a lactone group, the loss of the ethylenic group in C-7 position, and the presence of a sugar moiety. HMBC correlations from the methyl protons H-13 with quaternary carbon C-5 (δ_C 84.2), C-3 and C-4, and from methyl protons H-11 with carbons C-5, C-3, C-12, and C-6 (δ_C 53.3) provided compelling evidence for the cyclised form. Correlations in COSY between H-7 and its vicinal H-6 and H-8, between H-8 and its vicinal H-7 and H-9, and between H-9 and its vicinal H-8 and H-10 justified their respective positions. The stereochemistry of carbons C-1 and C-6 was established by the ROESY correlations between H-12 with H_{eq}-2, and H-6, alpha oriented, and between methyl H-11 with protons H-7b and H-8b. The chemical shift of C-9 (δ_H 3.91/δ_C 74.5) indicate a carbon bearing a hydroxyl and is indicative for 9*S* (ca. 74 ppm) configuration as observed for Δ7,8-type of 9-hydroxymegastigmane 9-*O*-*β*-D-glucopyranoside like in byzantionoside B (**19**) (Matsunami et al., 2010). The remaining ^1H and ^{13}C NMR resonances were assignable to two sugar moieties. The signals for an anomeric proton at δ_H 4.33 (1H, d, J = 7.8 Hz, H-1′), two oxymethylene protons at δ_H 3.59 (1H, dd, J = 11.9, 5.4 Hz) and 3.96 (1H, dd, J = 11.9, 2.2 Hz) (H_2-6′), an oxymethylene carbon at δ_C 67.3 (C-6′), five oxymethine carbons at δ_C 70.5 (C-4′), 73.7 (C-2′), 75.9 (C-5′), 76.8 (C-3′) and 100.9 (C-1′), and proton coupling constants above 7 Hz, were consistent with those of a *β*-glucopyranosyl moiety (Table 1). The large coupling constants from anomeric proton

(>7 Hz) and the axial correlations observed between H-1′/H-3' and H-1′/H-5' in the ROESY spectrum, indicated their *β*-configurations. A second anomeric proton at δ_H 5.07 (1H, d, *J* = 2.6 Hz, H-1") was correlated in COSY with an oxymethylene protons at δ_H 3.89 (1H, d, *J* = 2.6 Hz, H_{β}-2"), and the signals of two oxymethylenes carbon at δ_C 64.2 (C-5″) and 73.5 (C-4″), are consistent with an apiofuranose. HMBC correlations from H-9 to the anomeric carbon C-1' (δ_C 100.9) and from H-1" (δ_H 5.07) to the carbon C-6' indicate the positions of the sugar chain and the interglycosidic linkage. This is confirmed by the ROESY correlations between H-1′/H-9 and H-1"/H-6'. Thus, compound **18** was elucidated as **(**1*S*,6*R*,9*R*)-6-[3-hydroxybutyl]-1,5-dimethyl-12-oxabicyclo-[3.2.1]-octan-3-one-9-*O*-*α*-apiofuranoside-(1→6)-*O*-*β*-glucopyranoside name elaeisoside.

Compound **20** has the molecular formula $C_{24}H_{28}O_{13}$, deduced from the sodium molecular ion at *m/z* 499.1424 $[M + Na]^+$ (calcd $C_{20}H_{28}O_{13}Na$, 499.1428), in positive mode HR-ESI-MS. The ^1H NMR and ^{13}C NMR spectral data of compound **20** showed similarity to that of 2,3-dihydroxybenzoic acid methyl ester 3-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (Xu et al., 2009) (Table 1). Analysis of the COSY, ROESY, HSQC and HMBC spectra indicated that in contrast to the compound described by Xu et al, the second glucose unit is replaced by rhamnopyranose in **20**. It is characterised by its methyl group at δ_H 1.21 (3H, d, *J* = 6.2 Hz) and δ_C 16.5 (C-6"), and its anomeric proton at δ_H 4.71 (1H, d, *J* = 1.5 Hz) and carbon at δ_C 100.7 (C-1").The coupling constant of H-1" ($^3J_{H\text{-}1''/H\text{-}2''}$ = 1.5 Hz) and the correlations observed between H-1"/ H-2" in the ROESY spectrum of **20**, indicated the *α*-anomer configuration of the rhamnose unit. The structural elucidation of benzyl was readily established from the specific chemical shifts of an identified aromatic ring [$\delta_{H\text{-}5}$ 6. 91/$\delta_{C\text{-}5}$ 118.6, $\delta_{H\text{-}4}$ 7.39/$\delta_{C\text{-}4}$ 122.3, $\delta_{H\text{-}6}$ 7.57/$\delta_{C\text{-}6}$ 123.2] (Table 1). HMBC correlations from H-6 to δ_C 151.3 (C-2) and 170.4 (C-7), H-5 to δ_C 113.1 (C-1) and δ_C 145.7 (C-3) allowed their respective positioning. The ester function was justified by the HMBC crosspeak between C-7 and H_3-8 (δ_H 3.98/δ_C 51.6). The interglycosidic linkage was established by HMBC correlations from H-1' (δ_H 4.89) to carbon C-3 (δ_C 145.7) and from H-1" (δ_H 4.71) to carbon C-6' (δ_C 66.3) and by ROESY correlation between H-1"/H-6'. Thus, the structure of **20** was elucidated as methyl ester of 2,3-dihydroxybenzoic acid 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**20**) (Figs. 2 and 4).

2.2. Biological screening of crude extracts on T. gondii

The *n*-Hept, DCM, EtOAc and 80%MeOH extracts of the ten selected drugs were evaluated for their cytotoxic activity against Vero cells. Among the 40 tested extracts, six were found to be cytotoxic at the concentration of 25 µg/mL, namely the *n*-Hept extract of *T. coronatum,* the DCM extracts of *E. floribundus* and leaves of *O. ahia,* the EtOAc extracts of leaves and bark of *O. borealis* and the MeOH 80% extract of *O. Elatum* (Table 2). The toxicity induced by these extracts on cells leads to cell death, and therefore would have as an immediate consequence preventing cell invasion by tachyzoites or parasite multiplication. Consequently, very few parasites will be marked by antibodies in the enzyme-linked immunosorbent assay (ELISA), which will not reflect the actual inhibition of parasite

multiplication by the product. Therefore, only the non-cytotoxic extracts were then evaluated for their antitoxoplasmic activity *in vitro* at 25 µg/mL (Table 2). The results revealed that eight of the 34 non-cytotoxic extracts showed more than 60% parasite growth inhibition at 25 µg/mL. These extracts were the DCM and EtOAc extracts of *C. micranthum*, the MeOH 80% extract of *E. guineensis*, the *n*-Hept and DCM of *O. africana*, the EtOAc of the leaves and DCM of the stem bark of *O. ahia*, and the DCM extract of *T. coronatum* with a parasite growth inhibition rate of 79%, 64%, 94%, 67%, 87%, 91%, 96%, and 72%, respectively.

Previous study on *C. micranthum* leaves showed that its aqueous extract had moderate antitoxoplasmic activity with IC_{50} of 254 µg/mL (Benoit-Vical et al., 2000) and 217 µg/mL (Sepúlveda-Arias et al., 2015) against RH tachyzoites of *T. gondii* in the RMC5 cells. However, our work showed also that DCM and EtOAc extracts of this plant possessed antitoxoplasmic activity at 25 µg/mL on Vero cells with 79% ± 0.18, and 64% ± 0.23 growth inhibition, respectively. The use in traditional medicine of the genus *Omphalocarpum* as an antiparasitic is more highlighted in the literature. This fact has been corroborated by our results which supported the traditional use of the stem bark of *O. ahia* as an antiparasitic (Armah et al., 2016) based on the strong inhibition of *T. gondii* growth at 25 µg/mL of the DCM extracts from stem bark (96% ± 0.05) and EtOAc from leaves (91% ± 0.10). Whereas DCM extract of *O. elatum* was moderately active against *T. gondii* tachyzoites (57% ± 0.13). Our work showed for the first time that the stem bark of *Oldfieldia africana* and all plant of *Tristemma coronatum* were active against *T. gondii* at 25 µg/mL (Table 2). The traditional antiparasitic use of *E. guineensis* leaves in Côte d'Ivoire that had been observed for the 70% hydroethanolic extract against *P. falciparum* (another *Apicomplexa* parasite) with IC_{50} 1.195 µg/mL (Annan et al., 2012), was supported by the strong inhibition (94 % ± 0.07) of *T. gondii* growth at 25 µg/mL by the hydromethanolic 80% extract. This extract was therefore selected for further study to identify the compounds being involved in the biological effect against *T. gondii*.

2.3. Bioguided screening of MeOH extract of Elaeis guineensis on T. gondii

Based on the results of the antitoxoplasmic activity screening, an extraction was carried out on 1.5 kg of the air-dried and powdered *E. guineensis* leaves. The hydromethanolic extract was fractionated on a Diaion HP-20 resin column to obtain HP_1–HP_5 fractions. The cytotoxicity of the MeOH 80% extract and obtained fractions was evaluated at 25 µg/mL on Vero cells (Table 3) and showed that the less polar fraction HP_5 is cytotoxic with a growth inhibition rate of 67%. Therefore, HP_5 was excluded from the antiparasitic screening. The HP_1–HP_4 fractions were evaluated for their anti-*Toxoplasma* activity at the concentration 25 µg/mL, but none showed anti-*Toxoplasma* activity (Table 3). It seems that the fractionation of the hydromethanolic extract led to the loss of the antitoxoplasmic activity. A chemosensitivity on the HP_5 fraction was carried out to determine the concentration at which it is no longer cytotoxic, in order to measure its anti-toxoplasmic activity. The cytotoxicity of HP_5 was evaluated on Vero cells at 25, 12.5, 6.25, 3.125 and 1.563 µg/mL and the IC_{50} was found to be 16.0 µg/mL. The

concentration which induced less than 12 % of Vero cells growth inhibition was 6.25 µg/mL. Unfortunately, HP_5 showed less than 1% *T. gondii* growth inhibition at this concentration.

It is important to point out that, despite the historical effectiveness of bioguided fractionation of plant extracts, loss of activity and failure to isolate active compounds during this process can occur (Caesar and Cech, 2019; Nothias et al., 2018). The main reasons of these pitfalls may be the degradation of compounds during the purification process, low concentration of bioactive compounds making their isolation difficult, and/or potential synergistic effects (Nothias et al., 2018). This can be avoided by "synergy-directed fractionation" (Junio et al., 2011) which combines chromatographic separation and synergy testing in combination with a known active constituent in the original extract. Several studies have shown that the overall activity of plant extracts can result from mixtures of compounds with synergistic, additive, or antagonistic activity (Wagner and Ulrich-Merzenich, 2009).

While there are multiple possible explanations for this failure, it is true that in some cases loss of activity occurs because multiple constituents are required to observe the biological effect.

The first explanation could be the irreversible adsorption of compounds on the column packing (Qiu et al., 2013). To test this purpose, the MeOH 80% extract was fractionated by centrifugal partition chromatography (CPC), a solid support free liquid–liquid separation technique involving the distribution and transfer of solutes between at least two immiscible liquid phases according to their partition coefficients. This method avoids irreversible adsorption of analytes due to the absence of solid support. The CPC fractionation of the MeOH 80% extract led to eleven fractions which were evaluated for their cytotoxicity on Vero cells at 25 µg/mL showing that the less polar fractions (CPC_1–CPC_3) possessed cytotoxic activity (Table 3). The non-cytotoxic fractions were evaluated for their antiparasitic activity, unfortunately revealing that CPC_4–CPC_{11} fractions possessed less than 50% anti-*Toxoplasma* activity at 25 µg/mL. Thus, the loss of activity of the MeOH 80% extract is not due to irreversible adsorption of compounds to the column. This suggests that the anti-*Toxoplasma* activity of the MeOH 80% extract could result from mixtures of compounds with synergistic, additive, or antagonistic activity.

The second hypothesis was that there was a synergistic interaction between the metabolites of the MeOH 80% extract. It is known that when two or more drugs are given in combination, the effect can be superadditive (synergistic); that is, they can demonstrate action beyond that is expected of their individual power and effectiveness. Unlike synergy, some drug combinations may show sub-additivity (antagonism) or simple additivity (Tallarida, 2011). Thus, the idea that HP_5, the only cytotoxic fraction at 25 µg/mL, could be responsible of the anti-*Toxoplasma* activity of the MeOH 80% extract of *E. guineensis* at lower concentrations when mixed with another fraction was verified. So, five combinations were reconstituted $HP_{1A} + HP_5$, $HP_{1B} + HP_5$, $HP_2 + HP_5$, $HP_3 + HP_5$ and $HP_4 + HP_5$, respecting their ratios in the MeOH 80% extract (Table 4). These combinations were evaluated for their cytotoxicity at the concentration of 25 µg/mL on Vero cell line (Table 4) and, surprisingly, none of these combinations showed more than 20% cytotoxic activity at the concentration tested. The combination tested $HP_{1B} + HP_5$ (25 µg/mL), in which the HP_5 concentration was 13.88 µg/mL showed a toxicity of

19% while HP_5 tested alone showed toxicity of 40% at this concentration. The other combinations tested $HP_2 + HP_5$, $HP_3 + HP_5$, and $HP_4 + HP_5$ also showed that these combinations were less cytotoxic than HP_5 alone at the same concentration for HP_5 (Table 4). These results suggested a sub-additive (antagonist) effect concerning the cytotoxic activity on Vero cell line.

These five combinations were evaluated for their anti-*Toxoplasma* activity at the concentration 25 µg/mL. The $HP_3 + HP_5$ combination in which the concentration of HP_5 was 5.95 µg/mL showed an anti-*Toxoplasma* effect of 50%, resulting in synergistic activity since the anti-*Toxoplasma* activity of HP_5 alone at the same concentration was 1%. The most active combination was $HP_4 + HP_5$ with 74% antiparasitic activity (Table 4).

2.4. Discussion of the anti-toxoplasmic activity of compounds identified in E. guineensis

The activity observed in these plants could be attributed to a synergistic effect between the phytochemical constituents, including aliphatic acids, terpenoids, sterols and flavonoids which could have antiparasitic activities. However, further studies should be carried out to confirm and explain this synergistic or antagonistic effect between the identified compounds. All CPC fractions (CPC_1–CPC_{11}) were analyzed by ^1H and ^{13}C NMR spectroscopy in order to identify the main class of compounds (Table 3) and compared to HP_{1A}–HP_5 fractions. No additional compounds were identified.

HP_5 contained long-chain fatty acids identified as palmitic acid (**10**), palmitic acid methyl ester (**11**), elaidic acid methyl ester (**8**), and linoelaidic acid methyl ester (**9**). Three of them (**8-9** and **11**) belong to fatty acid methyl esters (FAMEs) which have been reported to exhibit antibacterial properties. Some authors have even described that FAMEs could be the next generation of antibiotic agents acting through membrane disruption and reactive oxygen species production (Casillas-Vargas et al., 2021). Fatty acids are a broad group of amphipathic molecules with a terminal carboxyl group that, based on the number of double bonds, are classified into saturated and unsaturated fatty acids. These molecules play a crucial role in the structure of cell membranes, energy production, signaling, and regulation of different biochemical pathways (De Carvalho and Caramujo, 2019). Most likely, fatty acids like as those in the active fraction HP_5 affected the lipid bilayer integrity or even the inner membrane complex of *T. gondii*. Both structures are vital for invasion and replication of the parasite (Choi et al., 2019). Similar compounds, such as hexadecanoic acid methyl ester, have been shown to affect the integrity of bacterial cell membranes (Shaaban et al., 2021). In addition to affecting cell membranes, inhibition of bacterial fatty acid synthesis is suggested to be linked to these molecules (Abubacker and Deepalakshmi, 2013, Zheng et al., 2005). *T. gondii* acyl-CoA diacylglycerol acyltransferase (TgDGAT) is an integral membrane protein localized in the cortical and perinuclear endoplasmic reticulum of the parasite and is responsible for triacylglycerol synthesis (Anacleto-Santos et al., 2023). Some studies have reported that oleate, palmitoleate, and linoleate impair parasite replication by interfering with TgDGAT function (Nolan et al., 2018). Therefore, *T. gondii* growth can be limited by controlling the parasite lipidomics, as previously reported (Anacleto-Santos et al., 2023; Smith et al., 2021). Based on these reports, the

fatty acids in HP_5 could disrupt the parasite's membranes or inhibit enzymes required for *T. gondii* fatty acid synthesis.

Like fatty acids, allyl glycoside such as erigeside F (**24**) isolated from HP_3 may also exhibit selective toxicity towards the parasite by inhibiting fatty acid synthesis within the parasite (enoyl-acyl carrier protein reductase).

Among drug discoveries with antibacterial potentials, 75% were terpenes, including mono, di- and triterpenes (Zacchino et al., 2017). Although the exact mechanism of action of terpenes remains unknown, it is believed that they can inhibit both oxygen uptake and oxidative phosphorylation processes (Grin et al., 1999). Terpenes have been extensively reported to exhibit antibiotic, antiviral, and antiparasitic properties (Bisht et al., 2021). The growth inhibitory activity of ursolic acid, a triterpenoid detected in HP_5, against *T. gondii* has already been reported with IC_{50} of 5.9 µM (Choi and Lee, 2019; Deng et al., 2019; Choi and Lee, 2018). Triterpenoids isolated from the barks of *Quercus crispula*, 29-norlupane-3,20-dione, oleanolic acid acetate, and ursolic acid acetate, showed anti-*Toxoplasma* activity with an IC_{50} between 6.8 and 24.4 µM (Endo et al., 2019). A pentacyclic triterpenoid from *Olea europaea*, maslinic acid, was evaluated on *T. gondii* tachyzoites and showed an IC_{50} of 58.2 µM. It seems that this terpenoid disrupts the ultrastructure and motility of the parasite due to inhibition of protease activity (De Pablos et al., 2010). Betulone, a lupane-type triterpene extracted from the bark of *Alnus glutinosa* showed anti-*Toxoplasma* activity *in vitro* (IC_{50} 2.7 µM) (Darme et al., 2021). Their ability to affect lipid membranes has also been reported (Mahizan et al., 2019). Some pentacyclic triterpenes have been shown to inhibit the expression of COX-2 (De-Almeida et al., 2019), which reduces *T. gondii* infection and upregulates the proinflammatory immune response in mice (Pereira et al., 2019). COX-2 is a crucial factor in *T. gondii* propagation in human trophoblasts; therefore, its inhibition can induce a proinflammatory response capable of controlling parasite proliferation (De Souza et al., 2021). Ursolic acid identified in HP_5 is also pentacyclic; therefore, it could affect the host Th1 immune response. A sterol, *β*-sitostenone, analogue of stigmasta-4,24(28)-dien-3-one (**15**) was evaluated on *T. gondii* tachyzoites and showed IC_{50} of 17.3 µM (Darme et al., 2022).

It has been demonstrated that a sesquiterpene, kurubasch aldehyde, exerts a moderate inhibitory effect on the growth of *Plasmodium falciparum* (IC_{50}=76 µM) and slow-proliferating breast cancer cells MCF7 (78 µM). However, it was discovered to be a highly potent inhibitor of S180 cancer cell proliferation, with an IC_{50} value of 7.4 µM (Traoré-Coulibaly et al., 2017). In a review published between 2015 and 2020, the authors conducted a comprehensive examination of the biological activity of sesquiterpenes and reported cytotoxicity, antibacterial, antifungal, anthelmintic and other activities. In light of these assertions, it would be useful to assess the anti-*Toxoplasma* activity of the nor-sesquiterpenes (**7** and **16-19**) identified in *E. guineensis*, from whitch three of previously undescribed, as the presence of glycoside could influence their pharmacological properties. The mode of action of terpenes includes, among other mechanisms, the generation of reactive oxygen species (ROS) and other free radicals after an oxidative stress process. Based on these facts, it could be inferred that the anti-*Toxoplasma* effect of *E. guineensis*

MeOH 80% extract could be linked to these mechanisms of action. Nevertheless, more studies need to be done in order to evaluate this extract as well as its possible characterized compounds on the complete life cycle of *T. gondii* and *in vivo* models.

T. gondii inhibits production of TNF-*α* and IL-12. TNF-*α* is a cytokine of inflammatory and immune response and together with IL-6 can enhance proliferation and differentiation of B lymphocytes (Lang et al., 2007). TNF-*α* activates eosinophil cytotoxicity toward protozoa and induces secretion of acute phase proteins via IL-6 production. TNF-*α* and IFN-*γ* have antiproliferative properties. In *toxoplasmosis,* TNF-*α* appears to be indispensable for macrophage activation and inhibition of parasite replication.

Several studies reported the anti-inflammatory properties of *E. guineensis* leaf extracts (Tow et al., 2021; Yin et al., 2013; Soundararajan and Sreenivasan, 2012). *E. guineensis* extracts contained flavonoids which have antioxidant, apoptosis-inducing, and anti-inflammatory properties (Hassanpour et al., 2023) and also decreases oxidative stress (Bubols et al., 2013; Miller, 1996). Flavonoid's mechanism is based on the activation of nuclear factor Nrf2 (Adetunji et al., 2023; Mendonca and Soliman, 2020), which activates other cellular defense mechanisms by Phase II initiation of detoxifying and antioxidant. However, there is a paucity of information in the literature regarding their cytotoxicity and anti-parasitic activity. Furthermore, *in vitro* studies have demonstrated that the combination of pyrimethamine with taxifolin (dihydroquercetin) exhibits significant inhibitory activity against *T. gondii* RH strain tachyzoites, with an IC_{50} of 1.39 µg/mL ($p < 0.05$). Notably, this combination is not cytotoxic for HFF and Hek-293 infected cell lines (Abugri et al., 2018). Similarly, Dégbé et al. (2018) demonstrated that the ethanolic extract of *Vernonia amygdalina* leaves exhibited a proportional content of phenols, tannins, flavonoids, and polysaccharides. The extract demonstrated the highest efficacy against the *T. gondii* RH strain tachyzoites and the lowest cytotoxicity on the HFF cell (Dégbé et al., 2018). These results suggest that the C-glycoside flavonoids isolated in the HP_3 fraction may modulate or enhance the antitoxoplasmic activity of terpenes, sterols, or other compounds in a mixture.

3. Conclusions

This study revealed the *in vitro* anti-*Toxoplasma* activities of the DCM and EtOAc extracts of the stem bark of *C. micranthum*, of the MeOH 80% extract of the leaves of *E. guineensis*, *n*-Hep and DCM extracts from the stem bark of *O. africana*, EtOAc extract from the leaves and DCM extract from the stem bark of *O. ahia*, from the DCM extract of the stem bark of *O. elatum* and the DCM extract of the aerial parts of *T. coronatum*. Further research will need to be investigated to identify their active components and to search for original compounds to treat toxoplasmosis.

Four undescribed compounds **16-18** and **20** and twenty known compounds were isolated from the leaves of *E. guineensis*. Among the compounds elucidated by dereplication or after purification, only vicenin-2 (**4**) and vitexin (**6**) were previously identified in *E. guineensis* by UHPLC-UV/PDA (Che Zain et al., 2021; Tow et al., 2021). The MeOH 80% extract of *E. guineensis* is active against *T. gondii* RH and is not cytotoxic to Vero cells at concentrations at 25 µg/mL that killed up to 94% of the parasites.

The fractionation of the MeOH 80% extract did not allow recovery of antitoxoplasmic activity in its individualized fractions. Its efficacy could be related to the mechanisms of action of fatty acids, flavonoids and terpenes; nevertheless, as different molecules are found in this extract, a synergistic or antagonistic effect cannot be discarded. It is therefore crucial to evaluate the components of the MeOH 80% extract as well as their mode of action, both in the parasite and host cells, to validate the vital targets (preferably those not supplied by the host) leading to the identification of a main molecule which will probably allow the design of analogous triterpenoids or FAMEs with anti-*Toxoplasma* activity. Finally, these results demonstrated that *E. guineensis* metabolites can be considered as possible candidates to obtain lead compounds against *T. gondii* and that natural compounds can be a good source for the development of new antiparasitic drugs. However, further biological studies will be required in order to promote the antiparasitic efficacy of these molecules.

4. Materials and methods

4.1. Instrumentation

1D and 2D-NMR spectra were recorded in CD_3OD on a Bruker Avance III 500 spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz) or on Bruker Avance DRX III 600 spectrometer equipped with a TCI cryoprobe, and using standard Bruker microprograms (Karlsruhe, Germany). The ^{13}C NMR spectra were acquired at 150.91 MHz using a standard zgpg pulse sequence with an acquisition time of 0.9 s, a relaxation delay of 3 s. HR-ESI-MS were obtained from Micromass Q-TOF micro-instrument (Manchester, UK). The centrifugal partition chromatography (CPC) equipment consists of a 303 mL FCPE300® column (Rousselet Robatel Kromaton, Annonay, France) containing 7 circular partition disks etched with a total of 231 partition twin cells (≈ 1 mL per twin cell), a Knauer Preparative 1800 V7115 pump (Berlin, Germany), a UVD 170 S detector set at 210, 254, 280 and 366 nm (Dionex, Sunnivale, CA, USA) and a Pharmacia Superfrac collector (Uppsala, Sweden). Flash chromatography was performed on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace cartridges (silica gel or RP-C_{18}), and the monitored wavelengths were at 205, 254, and 366 nm. Semi-preparative HPLC was performed on an apparatus equipped with an ASI-100 Dionex autosampler, an Ultimate 3000 pump ThermoFisher Scientific, a diode array detector UVD 340S and a Chromeleon software (Dionex, ThermoFisher Scientific, France). RP-C_{18} column (Phenomenex 250×15 mm, Luna 5 µm, Interchim, France) was used for the semi-preparative HPLC with a binary gradient eluent (H_2O pH 2.4 with TFA; CH_3CN) and a flow rate of 4.7 mL/min; RP-C_{18} column (Phenomenex 250×21,1 mm, Luna 5 µm, Interchim, France) was used for the preparative HPLC with a binary gradient eluent (H_2O pH 2.4 with TFA; CH_3CN) and a flow rate of 20 mL/min. The chromatogram was monitored at 205, 254, 300, and 360 nm. Analytical TLC was performed using silica gel plates (Merck Kieselgel 60 F254) and RP-C_{18} plates (Kieselgel 60 F_{254}) and visualized at 254 and 366 nm and by spraying the dried plates with vanillin and 50 % of H_2SO_4, followed by heating.

4.2. Chemicals, reagents, parasites and cell culture

All solvents for extraction, fractionation and solubilized were purchased from Carlo Erba Reactifs SDS (Val de Reuil, France). The RH strain (genotype I) of *T. gondii* was provided by the Toxoplasma Biological Resource Center (BRC Toxoplasma, France). Tachyzoites of *T. gondii* the RH strain were cultured on Vero cells monolayers (ATCC, CCL81) at 37 °C, 5% CO_2 in a humidified incubator. Cells and parasites were cultured in Iscove's Modified Dulbecco's Medium (IMDM) (Invitrogen, France) supplemented with 5% fetal bovine serum (SVF) for cells cultures or 2% (v/v) SVF for parasites cultures (Biowest, France) and supplemented with 1% antibiotics (penicillin-streptomycin) (GIBCO). All tested compounds were isolated by semi-preparative HPLC and compound purity was confirmed to be ≥ 95% prior to biological assays using analytical HPLC and quantitative NMR spectroscopy.

4.3. Plant material

The selected plants were all harvested in February 2021 in their natural habitats in Côte d'Ivoire. Botanical determination was performed by Mr Téré H.G. and authentication was made by Pr. Tiebre S.-M. from the National Floristic Centre (NFC) of university Félix HOUPHOUET-BOIGNY Cocody Abidjan (Côte d'Ivoire), in which, voucher specimens were deposited (Table 5).

4.4. Preparation of the 40 crude extracts

Air-dried plant material of each species (10 g) was ground (0.2 mm sieve). The plant material was extracted successively with *n*-Hept, DCM, EtOAc and MeOH 80%, at room temperature for 2 × 24 h, each 100 mL. The filtrates were taken to dryness under vacuum at 40 °C and the residues were stored at room temperature until testing.

4.5. Extraction and fractionation of E. guineensis leaves

Air-dried and powdered leaves of *E. guineensis* (1.5 kg) were extracted, at room temperature, for 2 × 24 h, with *n*-Hept, DCM, EtOAc and MeOH 80%, successively, each 15 L, to give after solvent removal under vacuum the corresponding extracts *n*-Hept (25.9 g), DCM (13.6 g), EtOAc (7.1 g) and MeOH 80% (44.6 g). A part of the MeOH 80% extract (30 g) was fractionated by Diaion HP-20 resine chromatography (5 × 45.5 cm), eluted successively with H_2O, 25%, 50%, 75% of MeOH in H_2O and finally with MeOH; each 3 L, to obtain fractions: HP_1 (20.5 g), HP_2 (1.5 g), HP_3 (3.8 g), HP_4 (2.5 g) and HP_5 (1.2 g), respectively. Fraction HP_I was solubilized in 200 mL of H_2O and partitioned with *n*-butanol (3 ×250 mL) to obtain the HP_{1A} (H_2O soluble fraction, 19.6 g) and HP_{1B} (*n*-BuOH soluble fraction, 0.9 g).

4.6. Chemical profiling of the six fractions by ^{13}C-NMR based dereplication

Briefly, fractions HP_1–HP_5 were chemically profiled based on ^{13}C NMR data as described in a previous work (Hubert et al., 2014; Darme et al., 2021). All ^{13}C NMR spectra of the fraction series were processed and submitted to Hierarchical Clustering Analysis (HCA) for the recognition of ^{13}C NMR metabolite fingerprints. In this way, similarity measurements between ^{13}C NMR signals belonging to individual structures within the fraction series were visualized as "chemical shift clusters" on a HCA correlation heat map given in Fig. 1. As a result, 9 major chemical shift clusters colored in red were revealed on the heat map, corresponding to the major metabolites of the MeOH 80% extract (Fig. 1). With the help of acd_lotus/nmrshiftdb2 (Nuzillard, 2023) and NP-MRD databases containing chemical shift values of natural metabolites, the corresponding chemical structures were identified.

4.7. Purification of HP_3 and HP_5 and identification of its major components

Fraction HP_3 (2 g) was separated into 16 subfractions named $HP_{3\text{-}1}$–$HP_{3\text{-}16}$ using silica gel flash chromatography in solid state mode and eluted with $CHCl_3$/MeOH/H_2O (from 95/5/0 to 70/30/5, v/v/v). Subfraction $HP_{3\text{-}2}$ (17 mg) was purified by semi-prep HPLC, eluted with a gradient from 90% to 100% of CH_3CN in water as a mobile phase, in 26 min to afford compounds **17** (t_R 21.0 min, 1 mg) and **19** (t_R 23.5 min, 1 mg). Subfraction $HP_{3\text{-}6}$ (17 mg) was purified under the same experimental conditions as subfraction $HP_{3\text{-}2}$ in semi-prep HPLC, to obtain compounds **23** (t_R 7.1 min, 1.5 mg) and **24** (t_R 20.0 min, 1.1 mg). Subfraction $HP_{3\text{-}8}$ (85 mg) was purified by preparative HPLC, eluted with a gradient from 90% to 100% of CH_3CN in water as a mobile phase, in 26 min to afford compound **7** (5.3 mg). The subfractions $HP_{3\text{-}10}$, $HP_{3\text{-}11}$, $HP_{3\text{-}14}$ $HP_{3\text{-}15}$ and $HP_{3\text{-}16}$ were purified under the same experimental conditions as subfraction $HP_{3\text{-}8}$ in preparative HPLC. Purification of subfraction $HP_{3\text{-}10}$ (233 mg) resulted in the isolation of compounds **18** (20.6 mg) and **20** (15.7 mg). Purification of subfraction $HP_{3\text{-}11}$ (382 mg) resulted in the isolation of compound **21** (24.3 mg). Purification of subfraction $HP_{3\text{-}14}$ (210 mg) resulted in the isolation of compound **5** (12.8 mg). Purification of subfraction $HP_{3\text{-}15}$ (266 mg) resulted in the isolation of compounds **22** (22.4 mg) and **6** (6.3 mg). Purification of subfraction $HP_{3\text{-}16}$ (302 mg) resulted in the isolation of compound **4** (16.4 mg).

Fraction HP_5 (1.1 g) was separated into 37 subfractions named $HP_{5\text{-}1}$–$HP_{5\text{-}37}$ using silica gel flash chromatography eluted with *n*-Hept/acetone/$CHCl_3$/MeOH (from 95/5/0/0 to 0/0/1/1, v/v/v/v). The combined subfractions $HP_{5\text{-}1}$ and $HP_{5\text{-}2}$ (21 mg) were purified by semi-prep HPLC, eluted with a gradient from 80% to 100% of CH_3CN in water as a mobile phase, in 10 min, then in isocratic elution with CH_3CN for 10 min to afford compounds **14** (t_R 12.9 min, 1 mg), **8** (t_R 15.1 min, 1 mg) and **9** (t_R 18.6 min, 1.3 mg). Subfraction $HP_{5\text{-}3}$ (20 mg) was purified by semi-prep HPLC, eluted with a gradient from 80% to 100% of CH_3CN in water as a mobile phase, in 5 min, then in isocratic elution with CH_3CN for 20 min to afford compounds **15** (t_R 16.5 min, 2.1 mg), **13** (t_R 17.2 min, 1 mg) and **12** (t_R 23.7 min, 1 mg). Subfraction $HP_{5\text{-}4}$ (13 mg) was purified by semi-prep HPLC, eluted with a gradient from 80% to 88% of CH_3CN in water as a mobile phase, in 10 min to afford compounds **10** (t_R 7.4 min, 1 mg) and **11** (t_R 8.9

min, 1 mg). Similarly, subfraction HP_{5-4} was purified by semi-prep HPLC, eluted with a gradient from 80% to 88% of CH_3CN in water as a mobile phase, in 7 min, then in isocratic elution with CH_3CN for 3 min at 88% of CH_3CN and in isocratic elution with CH_3CN for 5 min to afford compound **16** (t_R 3,3 min, 1 mg).

*4.7.1. (-)-(6S)-elaeisdione (**16**)*

yellowish solid; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 1; $^{20}[\alpha]_D$ -0.3° (*c* 0.1; MeOH), IR ν_{max} (cm^{-1}): OH (3357); CH_3 (2953-2823); 2x C=O (1713; 1669); HR-ESI-MS *m/z* 237.1125 $[M + H]^+$ (calcd $C_{13}H_{17}O_4$, 237.1127).

*4.7.2. elaeisdiol or 6-dehydroxy-cucubalactone (**17**)*

yellowish solid; UV (MeOH) λ_{max} nm (log ε): 220.0 (5. 00); $^{20}[\alpha]_D$ -5.0° (*c* 0.1; MeOH); ^1H NMR and ^{13}C NMR (CD_3OD), see Table 1; IR ν_{max} (cm^{-1}): OH (3550); CH_3 (2960-2870); COOC (1830); HR-ESI-MS *m/z* 223.1333 $[M - H_2O + H]^+$ (calcd $C_{13}H_{19}O_3$, 223.1312) or 205.1223 $[M - 2H_2O + H]^+$ (calcd $C_{13}H_{17}O_2$, 205.1226).

*4.7.3. elaeisoside (**18**)*

yellowish solid; UV (MeOH) λ_{max} nm (log ε): 280.0 (3. 11), 220.0 (5.00); $^{20}[\alpha]_D$ +25.2° (*c* 0.1; MeOH); ^1H NMR and ^{13}C NMR (CD_3OD), see Table 1; IR ν_{max} (cm^{-1}): OH (3371); CH_3 (2932-2910); C=O (1700); CH_2-O-C (1012); HR-ESI-MS *m/z* 543.2418 $[M + Na]^+$ (calcd $C_{24}H_{40}O_{12}Na$, 543.2417).

*4.7.4. 2,3-dihydroxybenzoic acid 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**20**)*

yellowish solid; UV (MeOH) λ_{max} nm (log ε): 278.0 (4. 82), 220.0 (5. 00); $^{20}[\alpha]_D$ -2,4° (*c* 0.1; MeOH); ^1H NMR and ^{13}C NMR (CD_3OD), see Table 1; IR ν_{max} (cm^{-1}): OH (3357); COOC (1673); CH_3 (2927); OH-phenol (1457); HR-ESI-MS *m/z* 499.1424 $[M + Na]^+$ (calcd $C_{20}H_{28}O_{13}Na$, 499.1428).

4.8. Centrifugal partition chromatography (CPC) fractionation of MeOH 80% extract

A three-phase solvent system composed of *n*-Hept/MtBE/CH_3CN/water in the ratio 1/1/1/1 (v/v/v/v) (500 mL, of each) was used. The solvent mixture was thoroughly equilibrated in a separatory funnel. After separation of the *n*-heptane-rich upper phase (UP_0), one equivalent volume of MTBE (500 mL) was added to the mixture of middle (MP_0) and lower (LP_0) phases in order to slightly reduce the polarity of the remaining two-phase solvent system. After decantation, the final middle and lower phases (MP and LP) were separated. The column was filled with the LP at the minimal rotation speed of 200 rpm. The rotation speed was then increased to 1400 rpm. The sample solution (1.5 g), dissolved in a mixture of LP/MP/UP_0 (45/10/5 v/v/v), was loaded into the column by progressively pumping the less polar UP_0 in the ascending mode. The flow rate of the mobile phase was then maintained at 10 mL/min until the end of the experiment. The UP_0 was pumped for 30 min after the release of the dead volume to ensure the elution of all hydrophobic compounds. The moderately polar MP was then pumped for 30 min to

elute compounds with a medium hydrophobicity. Finally, in order to recover the most hydrophilic compounds retained at the head of the column, the role of the two liquid phases was switched by pumping the aqueous LP as the mobile phase in the descending mode at 10 mL/min. Fractions of 20 mL were collected. The separation was monitored by UV at 210, 254, 280 and 366 nm. All fractions were analyzed by TLC and HPLC and then pooled, giving 11 fractions (CPC_1–CPC_{11}).

4.9. Solubilization of extracts and fractions

All extracts and fractions were solubilized in dimethyl sulfoxide (DMSO). The final DMSO concentration was less than 0.5% in the final mixture (DMSO/culture medium, v/v). DMSO is commonly used for *in vitro* and *in vivo* experiments and is an aprotic amphiphilic molecule containing a highly polar domain and two non-polar methyl groups (MacGregor, 1967). This characteristic allows it to be soluble in polar or non-polar solvents. This also makes it capable of solubilizing many organic compounds polar and non-polar (Balakin et al., 2004; Santos et al., 2003).

4.10. Cytotoxic activity

The *in vitro* cytotoxicity of all 40 crude extract, HP_1–HP_5 and CPC_1–CPC_{11} fractions was assessed on Vero cell cultures using the UptiBlue viable cell count assay (Interchim, France). The crude extracts were prepared at 250 µg/mL in dimethyl sulfoxide (DMSO)/culture medium, v/v, and kept at −30°C, for a final sample tested at 25 µg/mL. A suspension of 20 000 Vero cells were cultured in 96-well plate with IMDM supplemented with 5% SVF (v/v). After 4 hours of incubation, crude extracts or fractions to test were deposited in wells at 25 µg/mL. After 72 hours of incubation at 37°C and 5% CO_2, wells were washed with phosphate buffer saline and fresh IMDM culture medium supplemented with 5% SVF and 10% UptiBlue was added. After an additional 3 h of incubation, the plate was measured with spectrophotometer with two wavelength, 570 nm corrected at 600 nm (FLUOstar Omega microplate reader, BMG Labtech, France). A cytotoxicity threshold was arbitrarily defined at cell viability of 80%.

4.11. Anti-Toxoplasma activity

All the experiments were performed at least in triplicate. The crude extracts were prepared at 250 µg/mL in dimethyl sulfoxide (DMSO)/culture medium, v/v, and kept at −30°C, for a final sample tested at 25 µg/mL. 20,000 Vero cells were filled in 96-well plates with IMDM supplemented with 2% SVF (v/v), then incubated for 3 hours at 37°C and 5% CO_2. Then, 50,000 parasites of the RH strain contained in 50 µL of culture medium were deposited, followed by 3 h of incubation in the same conditions. This time allowed the parasites to sediment and invade the Vero cells. Host cells were infected in a parasite-to-cell ratio of 5:2. Wells were then washed with PBS and 100 µL and 25 µL of sample solution was deposited per well in duplicate to obtain a final concentration of 25 µg/mL or 100 µg/mL. Dilutions were performed with culture medium. Finally, the plates were incubated for 72 h at 37 °C and 5% CO_2

627 before being fixed with cold methanol for 5 min at −20 °C and then parasite multiplications were
628 revealed by enzyme immunoassay.
629 Of the 96 wells, eight wells were reserved as a control of the Vero alone representing 100% cell viability,
630 then six reserved wells were inoculated with *T. gondii* as a parasite growth control under untreated
631 conditions, and finally, two final wells were inoculated under the same conditions as described above
632 with pyrimethamine at 1 µg/mL as a control.
633 The IC_{50} assays were processed in the same conditions as previously described (Darme et al., 2022).
634 Cells were then rehydrated with 100 µL of PBS for 10 minutes at room temperature. The plate was
635 treated with 100 µL of a 3% H_2O_2 solution for 10 minutes at 37°C to destroy the endogenous peroxidases
636 of the Vero cells and then was washed with PBS and filled with 60 µL of primary polyclonal anti-*T.*
637 *gondii* antibody (Bio-rad Rabbit Anti-*T. gondii* 9070-0556) diluted to $1/1000^{th}$ in a conjugate buffer.
638 After 1 hour of incubation at 37 °C, wells were washed four times with wash buffer. Subsequently, 60
639 µL of secondary goat antibody anti-rabbit IgG coupled with horseradish peroxidase (Bio-rad Goat Anti-
640 Rabbit IgG (H + L) HRP conjugate 170-6515) diluted to $1/2000^{th}$ in the conjugate buffer were added,
641 and incubated for 1 hour at 37°C. The wells were washed four more times and 200 µL of OPD (*o-*
642 *phenylenediamine dihydrochloride*) substrate (P8412 Sigma-Aldrich, France) was added in each well.
643 After 15 min at room temperature protected from light, the enzymatic reaction was stopped by the
644 addition of 50 µL of hydrochloric acid (HCl) 3 M. To finish, 200 µL of each well were transferred to a
645 new well and a spectrophotometer reading was performed at 492 nm, corrected at 630 nm.
646 Each experiment was performed with three controls: control with host cells alone, control with host cells
647 and parasites without the drug for parasite multiplication, and control with host cells with parasites and
648 pyrimethamine at 1 µM. The intensity of the staining is proportional to the amount of parasites present
649 in the well.
650 The anti-*Toxoplasma* activity of samples was determined using the formula: $100 - [\Delta_{Ods}/ \Delta_{Odv}) \times 100]$;
651 Δ_{Odv} = OD at 492 nm – OD at 630 nm for Vero cells invaded by tachyzoites without sample; Δ_{Ods} = OD
652 at 492 nm – OD at 630 nm for Vero cells invaded by tachyzoites and treated with sample.
653
654 *4.12. Statistical analysis*
655 Statistical significance in plaque assay, proliferation, and fungal growth inhibition assay was evaluated
656 by two-tailed unpaired tests using an Excel spreadsheet. Statistical data are expressed as the mean value
657 ± standard error.
658
659 **Appendix A. Supplementary data**
660 The following items are available online at
661 Supplemental Material File 1
662
663

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Conflict of Interest

The authors declare that they have no conflicts of interest associated with this publication.

CRediT authorship contribution statement

NO: Data curation, Investigation, Methodology, Writing – original draft, Software. **MIT**: Investigation. **AAM**: Methodology, Supervision, Writing – original draft, Writing – review & editing. **SEB**: Data curation, Investigation, Validation, Supervision. **PAYK**: Formal analysis, Funding acquisition, Resources, Supervision. **IV**: Funding acquisition, Project administration, Resources, Supervision. **LVN**: Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

References

Abbiw, D.K., 1990. Useful plants of Ghana. West African uses of wild and cultivated plants. Intermediate Technology Publications, London and Royal Botanic Gardens, Kew, 154-157. https://doi.org/10.3362/9781780443737.

Abubacker M.N., Deepalakshmi T., 2013. *In vitro* antifungal potentials of bioactive compound methyl ester of hexadecanoic acid isolated from *Annona muricata* Linn. (Annonaceae) leaves. Biosci Biotechnol Res Asia, 10, 879–884. http://dx.doi.org/10.13005/bbra/1211.

Abugri, D. A., Witola, W. H., Russell, A. E., & Troy, R. M., 2018. *In vitro* activity of the interaction between taxifolin (dihydroquercetin) and pyrimethamine against Toxoplasma gondii. Chemical Biology & Drug Design, *91*(1), 194-201. https://doi.org/10.1111/cbdd.13070.

Adetunji J.A., Fasae K.D., Awe A.I., Paimo O.K., Adegoke A.M., Akintunde J.K., Sekhoacha M.P., 2023. The protective roles of citrus flavonoids, naringenin, and naringin on endothelial cell dysfunction in diseases. Heliyon, 9(6), e17166. https://doi.org/10.1016/j.heliyon.2023.e17166.

Agrawal P.K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry. 31(10):3307-30. https://doi.org/10.1016/0031-9422(92)83678-R.

Ahoua, A.R.C., Konan, A.G., Bonfoh, B., Koné, M.W., 2015. Antimicrobial potential of 27 plants consumed by chimpanzees (Pan troglodytes verus Blumenbach) in Ivory Coast. BMC Complement Altern Med. *15*(1), 383. https://doi.org/10.1186/s12906-015-0918-7.

Alake J., Aktey S.A., Ahlidja W., Armah F.A., Amponsah I.K., 2021. Indigenous knowledge affords antimicrobial plants for the management of infections. Int J Trop Dis Health. 42(3): 12-21. DOI: 10.9734/IJTDH/2021/v42i330441.

Alexandri E., Ahmed R., Siddiqui H., Choudhary M.I., Tsiafoulis C.G., Gerothanassis I.P., 2017. High resolution NMR spectroscopy as a structural and analytical tool for unsaturated lipids in solution. Molecules. 5;22(10):1663. doi: 10.3390/molecules22101663.

Anacleto-Santos, J., Calzada, F., López-Camacho, P. Y., López-Pérez, T. de J., Carrasco-Ramírez, E., Casarrubias-Tabarez, B., Fortoul, T. I., Rojas-Lemus, M., López-Valdés, N., & Rivera-Fernández, N., 2023. Evaluation of the anti-*Toxoplasma gondii* efficacy, cytotoxicity, and GC/MS profile of *Pleopeltis crassinervata* active subfractions. *Antibiotics*, *12*(5), 889;https://doi.org/10.3390/antibiotics12050889.

Annan, K., Sarpong, K., Asare, C., Dickson, R., Amponsah, KI., Gyan, B., Ofori, M., & Gbedema, SY., 2012. In vitro anti-plasmodial activity of three herbal remedies for malaria in Ghana : *Adenia cissampeloides* (Planch.) Harms., *Termina liaivorensis* A. Chev, and *Elaeis guineensis* Jacq. Pharmacognosy Res. *4*(4), 225–229. https://doi.org/10.4103/0974-8490.102270.

Armah F.A., Annan K., Dickson R.A., Mensah A.Y., Ameyaw E.O., Anning A.A., 2016. Anti-leishmanial, anti-inflammatory and antioxidant potential of *Omphalocarpum ahia* A. Chev. Der Pharm Lett. 8 (2):161-168.

Baatz, H., Mirshahi, A., Puchta, J., Gümbel, H., & Hattenbach, L.-O., 2006. Reactivation of Toxoplasma Retinochoroiditis Under Atovaquone Therapy in an Immunocompetent Patient. Ocular Immunology and Inflammation, 14(3), 185-187. https://doi.org/10.1080/09273940600659740.

Atindehou K.K., Koné M., Terreaux C., Traore D., Hostettemann K., Dosso M., 2002. Evaluation of the antimicrobial activity of medicinal plants from the Ivory Coast. Phytother Res. 16, 497–502. https://doi.org/10.1002/ptr.970.

Balakin, K. V., Ivanenkov, Y. A., Skorenko, A. V., Nikolsky, Y. V., Savchuk, N. P., & Ivashchenko, A. A., 2004. In Silico Estimation of DMSO Solubility of Organic Compounds for Bioscreening. SLAS Discovery, *9*(1), 22-31. https://doi.org/10.1177/1087057103260006.

Banfi S., Caruso E., Orlandi V., Barbieri P., Cavallari S., Vigano P., Clerici P., Chiodaroli L. 2014. Antibacterial activity of leaf extracts from *Combretum micranthum* and *Guiera senegalensis* (Combretaceae). Res J Microbiol. 9: 66–81. DOI: 10.3923/jm.2014.66.81.

Benoit-Vical F., Santillana-Hayat M., Kone-Bamba D., Mallie M., Derouin F. 2000. Anti- *Toxoplasma* activity of vegetal extracts used in West African traditional medicine. Parasite, *7*(1), 3-7. https://doi.org/10.1051/parasite/2000071003.

Berinyuy F., Ngoupayo J., Kwetche P.R.F., Kuate T.T., Kuamouo J., Fokam M.A., Tchaleu B.N., 2014. Phytochemical screening and antimicrobial activity of extracts from the stem bark of *Oldfieldia africana* Benth & Hook (Euphorbiaceae) : Preliminary study. Int.J.Curr.Res.Chem.Pharma.Sci.1(7): 101-109.

Bigna, J. J., Tochie, J. N., Tounouga, D. N., Bekolo, A. O., Ymele, N. S., Youda, E. L., Sime, P. S., & Nansseu, J. R., 2020. Global, regional, and country seroprevalence of Toxoplasma gondii in pregnant women : A systematic review, modelling and meta-analysis. Scientific Reports, *10*(1), 12102. https://doi.org/10.1038/s41598-020-69078-9.

Bisht B.S., Bankoti H., Bharti T., 2021. A review on therapeutic uses of terpenoids. J Drug Deliv Ther. 11, 182–185. https://doi.org/10.22270/jddt.v11i1-s.4523.

Bonouman-Ira, BOSSON-VANGA AH, OFFIANAN AT, ANGORA KE, & DJAMAN AJ., 2017. Séroprévalence de la toxoplasmose chez les femmes enceintes reçues au laboratoire de parasitologie de l'Institut Pasteur de Côte d'Ivoire. Seroprevalence of Toxoplasmosis among pregnant in the Laboritory of Institut Pasteur of Cote d'Ivoire. *Rev int sc méd -RISM*, 19(4), 267-271.

Bosch-Driessen L.E.H., Berendschot T.T.J.M., Ongkosuwito J.V., Rothova A., 2002. Ocular toxoplasmosis. Ophthalmology 109 (5) 869–878, https://doi.org/ 10.1016/S0161-6420(02)00990-9.

Bubols G.B., Vianna Dda R., Medina-Remon A., von Poser G., Lamuela-Raventos R.M., Eifler-Lima V.L., Garcia S.C., 2013. The antioxidant activity of coumarins and flavonoids. Mini Rev Med Chem., 13(3):318-34. doi: 10.2174/138955713804999775.

Burkill, H.M. 1985. The useful plants of West Africa, 2nd ed. Royal Botanic Garden. 458–459.

Burkill H.M., 1997. The useful plants of West Tropical Africa, 2nd ed.vol.3 Families J–L. *Royal Botanic Garden*s, *Kew-London*.

Caesar, L.K., Cech, N.B., 2019. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat. Prod. Rep. 36, 869–888. https://doi.org/10.1039/ c9np00011a.

Casillas-Vargas G., Ocasio-Malavé C., Medina S., Morales-Guzmán C., Del Valle R.G., Carballeira N.M., Sanabria-Ríos D.J., 2021. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Prog. Lipid Res. 82, 101093.Innes J.K., Calder P.C. (2018). Omega-6 fatty acids and inflammation. Prostaglandins Leukot. Essent. Fat. Acids, 132, 41–48. https://doi.org/10.1016/j.plipres.2021.101093.

Che Zain M.S., Osman M.F., Lee S.Y., Shaari K., 2021. UHPLC-UV/PDA method validation for simultaneous quantification of luteolin and apigenin derivatives from *Elaeis guineensis* leaf extracts: An application for antioxidant herbal preparation. Molecules. 19;26(4):1084. doi: 10.3390/molecules26041084.

Cheng, Y.-X., Zhou, J., Deng, S.-M., & Tan, N.-H, 2002. New Norsesquiterpenoids from Cucubalus baccifer. Planta Medica, 68(1), 91-94. https://doi.org/10.1055/s-2002-20054.

Choi J.-W., Lee J., Lee J.-H., Park B.-J., Lee E.J., Shin,S., Cha G.-H., Lee Y.-H., Lim, K., Yuk J.-M., 2019. Omega-3 polyunsaturated fatty acids prevent *Toxoplasma gondii* infection by inducing autophagy *via* AMPK activation. Nutrients 11, 2137. https://doi.org/10.3390/nu11092137.

Choi W.H., Lee I.A., 2018. Evaluation of anti-*Toxoplasma gondii*e ffect of ursolic acid as anovel Toxoplasmosis inhibitor. Pharmaceuticals 11(2), 43. https://doi.org/10.3390/ph11020043.

Choi W.H., Lee I.A., 2019. The mechanism of action of ursolic acid as a potential anti-Toxoplasmosis agent, and its immunomodulatory effects. Pathogens 8 (2), 61. https://doi.org/10.3390/pathogens8020061.

Codaccioni, C., Picone, O., Lambert, V., Maurice, P., Pomar, L., Winer, N., Guibaud, L., Lavergne, R., Saliou, A., Quinio, D., Benachi, A., Noel, C., Ville, Y., Cuillier, F., Pomares, C., Ferret, N., Filisetti, D., Weingertner, A., Vequeau-Goua, V., … Mandelbrot, L., 2020. Ultrasound features of fetal toxoplasmosis : A contemporary multicenter survey in 88 fetuses. *Prenatal Diagnosis*, *40*(13), 1741-1752. https://doi.org/10.1002/pd.5756

Darme P., Escotte-Binet S., Cordonnier J., Remy S., Hubert J., Sayagh C., Borie N., Villena I., Voutquenne-Nazabadioko L., Dauchez M., Baud S., Renault J.H., Aubert D., 2022. Anti-*Toxoplasma gondii* effect of lupane-type triterpenes from the bark of black alder (*Alnus glutinosa*) and identification of a potential target by reverse docking. Parasite. 29:7. doi: 10.1051/parasite/2022008.

Darme P., Spalenka J., Hubert J., Escotte-Binet S., Debelle L., Villena I., Sayagh C., Borie N., Martinez A., Bertaux B. 2021. Investigation of antiparasitic activity of ten European tree bark extracts on *Toxoplasma gondii* and bioguided identification of triterpenes in *Alnus glutinosa* barks. Antimicrob. Agents Chemother. 66, e01098-21. doi: 10.1128/AAC.01098-21.

De-Almeida S.C.X., da-Silva Â., Sousa N.R.T., Amorim I.H.F., Leite B.G., Neves K.R.T., Costa J.G.M., Felipe C.F.B., de-Barros Viana G.S., 2019. Antinociceptive and anti-inflammatory activities of a triterpene-rich fraction from *Himatanthus drasticus*. Braz. J. Med. Biol. Res.,52, e7798. https://doi.org/10.1590/1414-431X20197798.

De Carvalho C.C.C.R., Caramujo M.J., 2018. The various roles of fatty acids. Molecules, 23, 2583. https://doi.org/10.3390/molecules23102583.

Deng Y., Wu T., Zhai S.-Q., Li C.-H., 2019. Recent progress on anti-*Toxoplasma* drugs discovery: Design, synthesis and screening. Eur J Med Chem 183, 111711. https://doi.org/10.1016/j.ejmech.2019.111711.

De Pablos L.M., González G., Rodrigues R., García Granados A., Parra A., Osuna A., 2010. Action of a Pentacyclic Triterpenoid, Maslinic Acid, against *Toxoplasma gondii*. J. Nat. Prod., 73, 831–834. DOI: 10.1021/np900749b.

De Souza G., Silva R.J., Milián I.C.B., Rosini A.M., de Araújo T.E., Teixeira S.C., Oliveira M.C., Franco P.S., da Silva C.V., Mineo J.R., et al., 2021. Cyclooxygenase (COX)-2 modulates *Toxoplasma gondii* infection, immune response and lipid droplets formation in human trophoblast cells and villous explants. Sci. Rep., 11, 12709. https://doi.org/10.1038/s41598-021-92120-3.

Dégbé, M., Debierre-Grockiego, F., Tété-Bénissan, A., Débare, H., Aklikokou, K., Dimier-Poisson, I., & Gbeassor, M., 2018. Extracts of *Tectona grandis* and *Vernonia amygdalina* have anti- *Toxoplasma* and proinflammatory properties *in vitro*. *Parasite*, *25*, 11. https://doi.org/10.1051/parasite/2018014.

Derouin, F., 2001. Anti-toxoplasmosis drugs. *Current Opinion in Investigational Drugs (London, England*, *2*(10), 1368-1374. https://europepmc.org/article/MED/11890349

Diehl M.S., Atindehou K.K., Téré H., Betschart B., 2004. Prospect for anthelminthic plants in the Ivory Coast using ethnobotanical criteria. J Ethnopharmacol. 95, 277–284. https://doi.org/10.1016/j.jep.2004.07.012.

Dujovne, C. A., Chan, C. H., Zimmerman, H. J., 1967. Sulfonamide hepatic iInjury. N Engl J Med . *277*(15), 785–788. https://doi.org/10.1056/NEJM196710122771503.

Ebong, P. E., Owu, D. U., Isong, E. U., 1999. Influence of palm oil (*Elaesis guineensis*) on health. *Plant Foods Hum Nutr. 53*(3), 209–222. https://doi.org/10.1023/A:1008089715153.

Endo, M., Shigetomi, K., Mitsuhashi, S., Igarashi, M., & Ubukata, M., 2019. Isolation, structure determination and structure–activity relationship of anti-toxoplasma triterpenoids from Quercus crispula Blume outer bark. *Journal of Wood Science*, *65*(1), 3. https://doi.org/10.1186/s10086-019-1782-8.

Ferrea, G., Canessa A., Sampietro F., Cruciani M., Romussi G., Bassetti D., 1993. *In vitro* activity of a *Combretum micranthum* extract against herpes simplex virus types 1 and 2. Antiviral Res. 21 (4), 317–325. doi.org/10.1016/0166-3542(93)90010-G.

Filardi, F.L.R. (2018). Brazilian Flora 2020 : Innovation and collaboration to meet target 1 of the global strategy for plant conservation (GSPC). *Rodriguésia*, *69*(4), 1513–1527. https://doi.org/10.1590/2175-7860201869402.

Frenkel, J. K., 1974. Breaking the transmission chain of *Toxoplasma* : A program for the prevention of human toxoplasmosis. Bulletin of the New York Academy of Medicine, *50*(2), 228-235.

Grin, S.G., Wyllie, J.L., Markham, D.N., 1999. Leach, The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour. Fragr. J. 14, 322–332. https://doi.org/10.1002/(SICI)1099-1026 (199909/10)14:53.0.CO;2-4.

Hamerski, L., Bomm, M. D., Silva, D. H. S., Young, M. C. M., Furlan, M., Eberlin, M. N., Castro-Gamboa, I., Cavalheiro, A. J., & da Silva Bolzani, V., 2005. Phenylpropanoid glucosides from leaves of Coussarea hydrangeifolia (Rubiaceae). Phytochemistry, 66(16), 1927-1932. https://www.sciencedirect.com/science/article/pii/S0031942205002943.

Hassanpour S.H., Doroudi A. (2023). Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed.,13(4):354-376. doi: 10.22038/AJP.2023.21774.

Holland G.N., 2004. Ocular toxoplasmosis: a global reassessment: part II: disease manifestations and management, Am J Ophthalmol. 137 (1), 1–17, https://doi.org/10.1016/j.ajo.2003.10.032.

Horvath, G., Wessjohann, L., Bigirimana, J., Jansen, M., Guisez, Y., Caubergs, R., & Horemans, N., 2006. Differential distribution of tocopherols and tocotrienols in photosynthetic and non-photosynthetic tissues. Phytochemistry, *67*(12), 1185-1195. https://doi.org/10.1016/j.phytochem.2006.04.004.

Hu Y., Liu X., Wu X., Zhang Z., Wu D., Chen C., Su W., Zhang L., Li J., Wang H.D., 2022. Several natural phytochemicals from Chinese traditional fermented food-pickled *Raphanus sativus L.*: Purification and characterization. Food Chem X. 16;15:100390. doi: 10.1016/j.fochx.2022.100390.

Hubert, J., Nuzillard, J.-M., Purson, S., Hamzaoui, M., Borie, N., Reynaud, R., Renault, J.-H., 2014. Identification of natural metabolites in mixture : A pattern recognition strategy based on ^{13}C NMR. *Anal Chem. 86*(6), 2955–2962. https://doi.org/10.1021/ac403223f.

IIslam, M.N., Ishita I.J., Jung H.A., Choi J.S., 2014. Vicenin 2 isolated from *Artemisia capillaris* exhibited potent anti-glycation properties. Food Chem Toxicol. 69:55-62. doi: 10.1016/j.fct.2014.03.042.

Israelski, D.M., Remington J.S., 1993. Toxoplasmosis in patients with cancer. Clin Infect Dis. 17 (Suppl) S423–S435. https://doi.org/10.1093/clinids/17.Supplement_2.S423.

Ito N., Etoh T., Hagiwara H., Kato M., 1997, Novel synthesis of degradation products of carotenoids, megastigmatrienone analogs and blumenol-A. J. Chem. Soc. Perkin Transactions 1: Organic and Bio-Organic Chemistry 10. 1571-1579. DOI: 10.1039/A607028K.

Junio, H.A., Sy-Cordero, A.A., Ettefagh, K.A., Burns, J.T., Micko, K.T., Graf, T.N., Richter, S.J., Cannon, R.E., Oberlies, N.H., Cech, N.B., 2011. Synergy-directed fractionation of botanical medicines: a case study with goldenseal (hydrastis canadensis). J. Nat. Prod. 74, 1621–1629. https://doi.org/10.1021/np200336g.

Kim J.H., Lee B.C., Kim J.H., Sim G.S., Lee D.H., Lee K.E., Yun Y.P., Pyo H.B., 2005. The isolation and antioxidative effects of vitexin from Acer palmatum. Arch Pharm Res.;28(2):195-202. doi: 10.1007/BF02977715.

Kim, H. M., Lee, D. G., & Lee, S. 2015. Plant-derived molecules from *Saussurea grandifolia* as inhibitors of aldose reductase. Journal of the Korean Society for Applied Biological Chemistry. *58*(3), 365–371. https://doi.org/10.1007/s13765-015-0051-9.

Kim S.J., Scott I.U., Brown G.C., Brown M.M., Ho A.C., Ip M.S., FRecchia .M., 2013. Interventions for *Toxoplasma retinochoroiditis.* Ophthalmology 120 (2), 371–378, https://doi.org/10.1016/j.ophtha.2012.07.061.

Koné M., Atindehou Kamanzi K., Traoré D., 2002. Plantes et médecine traditionnelle dans la région de Ferkessédougou (Côte d'Ivoire). West-African annals of botany, 2, 13–23.

Krajewski, D., Tóth, G., & Schreier, P., 1996. 2-Ethyl-3-methylmaleimide N-β-d-glucopyranoside from the leaves of mangosteen (Garcinia mangostana). Phytochemistry, 43(1), 141-143. https://doi.org/10.1016/0031 9422(96)00276-2.

Lang C., Groß U., Lüder C.G.K., 2007. Subversion of innate and adaptive immune responses by *Toxoplasma gondii*. Parasitology Research.100(2),191–203. DOI 10.1007/s00436-006-0306-9.

Liu Y., Abreu P.J.M., 2006. Long chain alkyl and alkenyl phenols from the roots of *Ozoroa insignis*. J Braz Chem Soc 17:527–532. https://doi.org/10.1007/s00044-016-1521-2.

Miller A., 1996. Antioxidant flavonoids: Structure, function and clinical usage. Altern Med Rev.1(2):103-111.

MacGregor, W. S., 1967. The chemical and physical properties of DMSO. *Annals of the New York Academy of Sciences*, *141*(1), 3-12. https://doi.org/10.1111/j.1749-6632.1967.tb34860.x.

Mahato, S.B. and Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids-A compilation and some salient features. Phytochemistry, 37,1517-1575. http://dx.doi.org/10.1016/S0031-9422(00)89569-2.

Mahizan N.A., Yang S.K., Moo C.L., Song A.A., Chong C.M., Chong C.W., Abushelaibi A., Lim S.E., Lai K.S., 2019. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules,24, 2631. https://doi.org/10.3390/molecules24142631.

Mamaghani, A. J., Fathollahi, A., Arab-Mazar, Z., kohansal, K., Fathollahi, M., Spotin, A., Bashiri, H., Bozorgomid, A., 2023. *Toxoplasma gondii* vaccine candidates : A concise review. Ir. J. Med. Sci. *192*(1), 231–261. https://doi.org/10.1007/s11845-022-02998-9.

Matsunami K., Otsuka H., Takeda Y., 2010. Structural revisions of blumenol C glucoside and byzantionoside B. Chem Pharm Bull.;58(3):438-41. doi: 10.1248/cpb.58.438.

McLeod R., Kieffer F., Sautter M., Hosten T., Pelloux H., 2009. Why prevent, diagnose and treat congenital toxoplasmosis? Mem Inst Oswaldo Cruz. 104 (2) (2009) 320–344, https://doi.org/10.1590/S0074-02762009000200029.

Mendonca P., Soliman K.F.A., 2020. Flavonoids activation of the transcription factor Nrf2 as a hypothesis approach for the prevention and modulation of SARS-CoV-2 infection severity. Antioxidants, 24;9(8):659. doi: 10.3390/antiox9080659.

Newman, D. J., Cragg, G. M., 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod*, 83*(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285.

Nguenang G.S., Mbaveng A.T., Fankam A.G., Manekeng H.T., Nayim P., Wamba B.E.N., Kuete V., 2018. *Tristemma hirtum* and five other Cameroonian edible plants with weak or no antibacterial effects modulate the activities of antibiotics against Gram-negative multidrug-resistant phenotypes. Sci World J, 22; 2018:7651482. doi: 10.1155/2018/7651482.

Nogareda, F., Strat, Y. L., Villena, I., De Valk, H., Goulet, V., 2014. Incidence and prevalence of *Toxoplasma gondii* infection in women in France, 1980–2020 : Model-based estimation. Epidemiol Infect. *142*(8), 1661–1670. https://doi.org/10.1017/S0950268813002756.

Nolan S.J., Romano J.D., Kline J.T., Coppens I., 2018. Novel approaches to kill *Toxoplasma gondii* by exploiting the uncontrolled uptake of unsaturated fatty acids and vulnerability to lipid storage inhibition of the parasite. Antimicrob Agents Chemother, 62, e00347-18. https://doi.org/10.1128/aac.00347-18.

Nothias, L.F., Nothias-Esposito, M., Da Silva, R., Wang, M., Protsyuk, I., Zhang, Z., Sarvepalli, A., Leyssen, P., Touboul, D., Costa, J., Paolini, J., Alexandrov, T., Litaudon, M., Dorrestein, P.C., 2018. Bioactivity-based molecular networking for the discovery of drug leads in natural product bioassay-guided fractionation. J. Nat. Prod. 81, 758–767. https://doi.org/10.1021/acs.jnatprod.7b00737.

Nuzillard, J.-M., 2023. Use of carbon-13 NMR to identify known natural products by querying a nuclear magnetic resonance database—An assessment. Magnetic Resonance in Chemistry, *61*(11), 582-588. https://doi.org/10.1002/mrc.5386.

Nyasangox T., Fru C.G., Sithole S., Mukanganyama S., 2018. The effects of phytosterols isolated from *Omphalocarpum elatum* (Sapotaceae) on mouse peritoneal cells. J Drug Discov Develop and Deliv, 5(1): 1032.

Osonwa U.E., Umeyor C.E., Okon J.E., Joel E. Agboke A., Udosen V.M., 2012. Physicotechnical properties of tablets formulated with ethanolic extract of fresh leaves of *Combretum micranthum* G. Don. J Applied Pharm Sci. 2 (12), 125–129, DOI: 10.7324/JAPS.2012.21223.

Pauli G.F., Junior P., 1995. Phenolic glycosides from *Adonis aleppica.* Phytochemistry, 38, 1245-1250, https://doi.org/10.1016/0031-9422(94)00635-7.

Pereira A.C.A., Silva R.J., Franco P.S., de Oliveira Gomes A., Souza G., Milian I.C.B., Ribeiro M., Rosini A.M., Guirelli P.M., Ramos E.L.P., 2019. Cyclooxygenase (COX)-2 inhibitors reduce *Toxoplasma gondii* infection and upregulate the pro-inflammatory immune response in *Calomys callosus* rodents and human monocyte cell line. Front. Microbiol, 10, 225. https://doi.org/10.3389/fmicb.2019.00225.

Petrelli, R., Orsomando, G., Sorci, L., Maggi, F., Ranjbarian, F., Biapa Nya, P. C., Petrelli, D., Vitali, L. A., Lupidi, G., Quassinti, L., Bramucci, M., Hofer, A., Cappellacci, L., 2016. Biological activities of the essential oil from *Erigeron floribundus. Molecules*, *21*(8), Article 8. https://doi.org/10.3390/molecules21081065.

Purser S., Moore P.R., Swallow S., Gouverneur V., 2008. Fluorine in medicinal chemistry, Chem. Soc. Rev. 37 (2), 237–432, https://doi.org/10.1039/ b610213c.

Qiu F., Cai G., Jaki B.U., Lankin D.C., Franzblau S.G., Pauli G.F., 2013. Quantitative purity-activity relationships of natural products: the case of anti-tuberculosis active triterpenes from *Oplopanax horridus.* J Nat Prod, **76**, 413–419. https://doi.org/10.1021/np3007809.

Roberts E., Eargle J., Wright D., Luthey-Schulten Z., 2006). MultiSeq: unifying sequence and structure data for evolutionary analysis, BMC Bioinf, 7, 382. https:// doi.org/10.1186/1471-2105-7-382.

Robert-Gangneux F., Aubert D., Villena I. Toxoplasmosis: a widespread zoonosis diversely affecting humans and animals. Editeur: A Sing (ed), Zoonoses: Infections affecting Humans and animals pour Springer, Cham., 2022, ISBN 978-3-030-85877-3, doi : 10.1007/978-3-030-85877-3_14-1

Rusaati B.I.W., Gendusa A.P., Joo S.H., Park J.W., Masumbuko N.C., Iragi K.G., Ngbolua K.-T.-N., Furaha M.A., Rolly N.K., Kang J.W., 2021. A systematic review of antimalarial medicinal plants in Democratic Republic of the Congo. Bois et Forêts des Tropiques, 347: 11-27. Doi : https://doi. org/10.19182/bft2021.347.a31882.

Sandjo L.P., Fru C.G., Kuete V., Nana F., Yeboah S.O., Mapitse R., Abegaz B.M., Efferth T., Opatz T., Ngadjui B.T., 2014. Elatumic acid: a new ursolic acid congener from *Omphalocarpum elatum* Miers (Sapotaceae). Z Naturforsch C J Biosci, 69(7-8):276-82. doi: 10.5560/znc.2014-0050.

Santos, N. C., Figueira-Coelho, J., Martins-Silva, J., & Saldanha, C., 2003. Multidisciplinary utilization of dimethyl sulfoxide : Pharmacological, cellular, and molecular aspects. Biochemical Pharmacology, *65*(7), 1035-1041. https://doi.org/10.1016/s0006-2952(03)00002-9.

Sepulveda-Arias J.C., Veloza L.A., Mantilla-Muriel L.E., 2014. Anti-*Toxoplasma* activity of natural products: a review. Recent Pat Anti infect Drug Discov,9(3):186-94. doi: 10.2174/1574891x10666150410120321.

Shaaban M.T., Ghaly M.F., Fahmi S.M., 2021. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. J Basic Microbiol*.,* 61, 557–568. doi: 10.1002/jobm.202100061.

Sheu J.H., Wang G.H., Sung P.J., Duh C.Y., 1999. New cytotoxic oxygenated fucosterols from the brown alga *Turbinaria conoides*. J Nat Prod.;62(2):224-7. doi: 10.1021/np980233s.

Smith N.C., Goulart C., Hayward J.A., Kupz A., Miller C.M., van Dooren G.G., 2021. Control of human toxoplasmosis. Int. J. Parasitol, 51, 95–121. https://doi.org/10.1016/j.ijpara.2020.11.001.

Soundararajan V., Sreenivasan S., 2012. Antioxidant activity of *Elaeis guineensis* leaf extract: An alternative nutraceutical approach in impeding aging. APCBEE Procedia, 2, 153 – 159. https://doi.org/10.1016/j.apcbee.2012.06.028.

Tallarida R.J., 2011. Quantitative methods for assessing drug synergism. Genes Cancer. 2(11), 1003-8. doi: 10.1177/1947601912440575.

Tepongning R.N., Mbah J.N., Avoulou F.L., Jerme M.M., Ndanga E.K., Fekam F.B., 2018. Hydroethanolic extracts of *Erigeron floribundus* and *Azadirachta indica* reduced *Plasmodium berghei* parasitemia in balb/c mice. Evid Based Complement Alternat Med. 21, 5156710. doi: 10.1155/2018/5156710.

Tomaz A.C.d.A., Miranda G.E.C.d., Souza M.d.F.V.d., da Cunha E.V.L., 2012. Analysis and characterization of methyl esters of fatty acids of some *Gracilaria* species. Biochem Syst Ecol, 44, 303–306. https://doi.org/10.1016/j.bse.2012.02.006.

Tow W.K., Goh A.P., Sundralingam U., Palanisamy U.D., Sivasothy Y., 2021. Flavonoid composition and pharmacological properties of *Elaeis guineensis* Jacq. leaf extracts: A systematic review. Pharmaceuticals (Basel). 24;14(10):961. doi: 10.3390/ph14100961.

Tra Bi F.H., Koné M.W., Kouamé N.F., 2008. Antifungal activity of *Erigeron floribundus* (Asteraceae) from Côte d'Ivoire, West Africa. Trop J Pharm Res, 7, 975–979. DOI: 10.4314/tjpr.v7i2.14681.

Traoré-Coulibaly, M., YOugBaRé, S., OuédRaOgO, J. B., Guissou, I. P., Nacoulma, O. G., Guiguemdé, T. R., & Christensen, S. B., 2017. Activité antiplasmodique de Canthium multiflorum (Schumach. & Thonn.) Hiern (Rubiacées) et de Trichilia emetica Vahl.(Méliacées) utilisées dans le traitement du paludisme. *Sciences de la Santé*, *40*(2).

Valnet, J., 1983. Traitement des maladies par les plantes. Ed. Maloine, Paris, pp. 500–512.

Wagner H., Ulrich-Merzenich G., 2009. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine, 16 , 97 - 110. https://doi.org/10.1016/j.phymed.2008.12.018.

Wong S.-Y., Remington J.S., 1994. Toxoplasmosis in pregnancy, Clin Infect Dis. 18 (6), 853–862, https://doi.org/10.1093/clinids/18.6.853.

Xu, M., Yang, C. R., & Zhang, Y. J., 2009. Minor antifungal aromatic glycosides from the roots of Gentiana rigescens (Gentianaceae). Chinese Chemical Letters, 20(10), 1215-1217. https://doi.org/10.1016/j.cclet.2009.05.006.

Yamamura, S., Ozawa, K., Ohtani, K., Kasai, R., & Yamasaki, K., 1998. Antihistaminic flavones and aliphatic glycosides from Mentha spicata. Phytochemistry, 48(1), 131-136. https://doi.org/10.1016/S0031 9422(97)01112-6.

Yan J., Shi X., Donkor P.O., Zhu H., Gao X., Ding L., Qiu F., 2017. Nine pairs of megastigmane enantiomers from the leaves of *Eucommia ulmoides* Oliver. *J Nat Med*, **7**1, 780–790. https://doi.org/10.1007/s11418-017-1102-9.

Yin N.S., Abdullah S.,Phin C.K., 2013. Phytochemical Constituents From Leaves of *Elaies Guineensis* and Their Antioxidants and Antimicrobial Activities. Int J Pharm Pharm Sci, Vol 5, Suppl 4, 137-140.

Zacchino, S.A., Butassi, E., Cordisco, E., Svetaz, L.A., 2017. Hybrid combinations containing natural products and antimicrobial drugs that interfere with bacterial and fungal biofilms. Phytomed. 37, 14–26. https://doi.org/10.1016/ j.phymed.2017.10.021.

Zheng C.J., Yoo J.-S., Lee T.-G., Cho H.-Y., Kim Y.-H., Kim W.-G., 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett. 579, 5157–5162. https://doi.org/10.1016/j.febslet.2005.08.028.

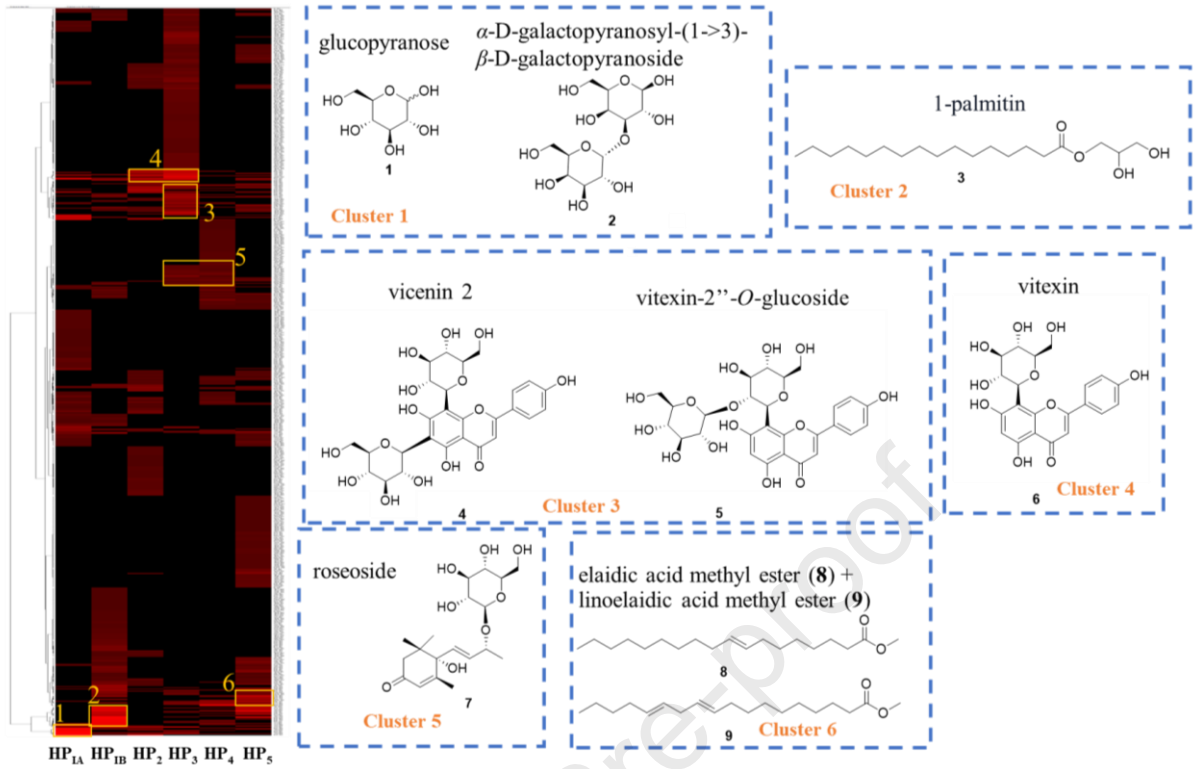


Fig. 1. Heatmap of peak intensities of ^{13}C NMR (rows) and fractions (columns) from the MeOH 80% extract of *E. guineensis*. This representation allows the visualization of the carbon skeleton of the major compounds.

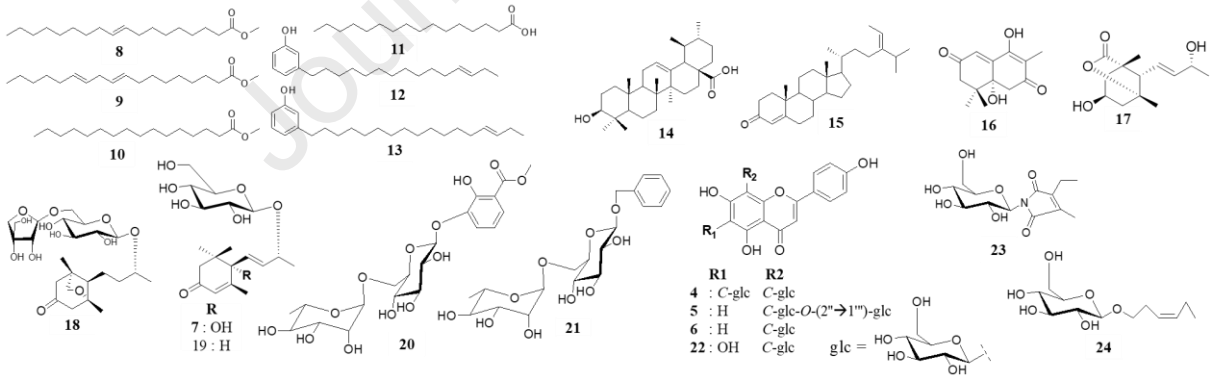


Fig. 2. Structures of compounds purified from HP_3 and HP_5 fractions of *E. guineensis.*

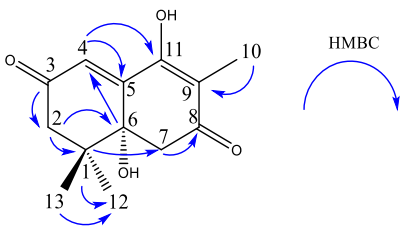


Fig. 3. Main HMBC correlations of compound **16**.

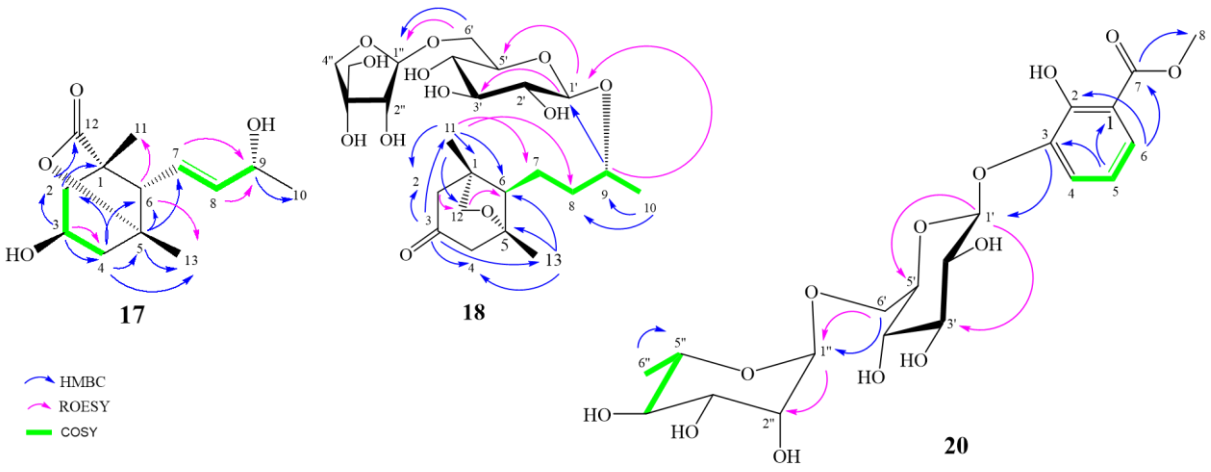


Fig. 4. Main HMBC, COSY and ROESY correlations of compounds **17-18** and **20**.

Table 1

^{13}C and ^{1}H-NMR spectroscopic data for compounds **16-18** and **20** in $CDCl_3$ or MeOH-d_4.[a, b].

Position	**16**		**17**		**18**		**20**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.0	-	46.2	-	43.4	-	113.1	-
2	39.9	3.07 d (18.4) 2.67 d (18.4)	35.8	1.62 dt (13.7, 6.5)^{eq} 1.76 dd (13.7, 7.3)^{ax}	48.2	2.18 dt (17.5, 1.9)^{ax} 2.59 d (17.5)^{eq}	151.3	-
3	201.0	-	64.5	3.80 m	211.2	-	145.7	-
4	131.9	6.95 br. s	37.5	1.64 dd (13.8, 6.8)^{eq} 2.11 dd (13.8, 6.8)^{ax}	49.2	2.23 dt (17.5, 1.9)^{ax} 2.61 d (17.5)^{eq}	122.3	7.39 dd (8.2, 1.5)
5	142.8	-	85.5	-	84.2	-	118.6	6.91 t (8.1)
6	83.8	-	57.5	2.49 d (9.6)	53.3	1.76 dd (6.7, 1.9)	123.2	7.57 dd (8.1, 1.5)
7	50.5	2.55 d (17.2) 2.44 d (17.2)	120.3	5.77 dd (15.4, 9.6)	20.8	1.52 m 1.90 m	170.4	-
8	195.7	-	142.4	5.87 dd (15.4, 5.4)	36.2	1.72 m 1.74 m	51.6	3.98 s
9	103.1	-	67.3	4.31 q (6.4)	74.5	3.91 m		
10	17.5	1.74 s	22.3	1.28 d (6.2)	18.9	1.24 d (6.2)		
11	160.0	-	17.8	1.10 s	19.5	1.11 s		
12	23.2*	1.26 s*	180.0	-	78.0	3.58		
13	21.9*	1.25 s*	22.2	1.36 s	23.8	1.34 s		
					glc		glc	
1’					100.9	4.33 d (7.8)	101.4	4.89 d (7.7)
2’					73.7	3.17 dd (9.3, 7.8)	73.4	3.54 m
3’					76.8	3.36 t (9.3)	76.3	3.48 m
4’					70.5	3.23 t (9.3)	70.1	3.40 m
5’					75.9	3.42 ddd (7.4, 5.4, 2.2)	76.7	3.36 m
6’					67.3	3.59 dd (11.9, 5.4) 3.96 dd (11.9, 2.2)	66.3	3.61 m 4.02 d (10.8, 1.6)
					api		rham	
1”					109.5	5.07 d (2.6)	100.7	4.71 d (1.5)
2”					76.6	3.89 d (2.6)	70.7	3.27 t (6.9)
3”					79.1	-	71.4	3.82 dd (3.4, 1.6)
4”					73.5	3.75 d (9.5) 3.95 d (9.5)	72.6	3.36 m
5”					64.2	3.59 m	68.4	3.64 m
6”							16.5	1.21 d (6.2)

[a] in ppm, *J* in brackets in Hz.

[b] NMR spectra recorded at 500 or 600 MHz (^{1}H) and at 125 or 150 MHz (^{13}C).

961 **Table 2**

962 *In vitro* cytotoxicity of selected plant extracts on Vero cells.

Plant species	Plant part	Extract	Cytotoxic activity against Vero cells	Anti-*toxoplasma* activity against *T. gondii*
C. micranthum	leaf	*n*-Hep	**3% ± 0.02**	1% ± 0.01
		DCM	**2% ± 0.01**	**79% ± 0.18**
		EtOAc	**3% ± 0.03**	**64% ± 0.23**
		MeOH 80%	**3% ± 0.04**	5% ± 0.04
E. guineensis	leaf	*n*-Hep	**2% ± 0.03**	29 % ± 0.16
		DCM	**2% ± 0.01**	1% ± 0.01
		EtOAc	**6% ± 0.06**	9% ± 0.09
		80% MeOH	**5% ± 0.04**	**94% ± 0.07**
E. floribundus	aerial parts	*n*-Hep	**3% ± 0.03**	13% ±0.13
		DCM	56% ± 0.9	nd[a]
		EtOAc	**3% ± 0.02**	25% ±0.18
		MeOH 80%	**4% ± 0.04**	3% ± 0.03
O. africana	stem bark	*n*-Hep	**10% ± 0.02**	**67% ± 0.08**
		DCM	**10% ± 0.05**	**87% ± 0.09**
		EtOAc	**3% ± 0.02**	7% ± 0.07
		MeOH 80%	**7% ± 0.03**	14% ± 0.09
O. ahia	leaf	*n*-Hep	**6% ± 0.03**	7% ± 0.03
		DCM	82% ±0.17	nd[a]
		EtOAc	**16% ±0.14**	**91% ± 0.10**
		MeOH 80%	**10% ± 0.04**	6% ± 0.08
O. ahia	Stem bark	*n*-Hep	**4% ± 0.02**	10% ± 0.09
		DCM	**7% ± 0.02**	**96% ± 0.05**
		EtOAc	**5% ± 0.07**	23% ± 0.17
		MeOH 80%	**3% ± 0.02**	1% ± 0.01
O. borealis	leaf	*n*-Hep	**7% ± 0.02**	11% ± 0.12
		DCM	**4% ± 0.03**	5% ±0.04
		EtOAc	32% ± 0.10	nd[a]
		MeOH 80%	**11% ± 0.06**	1% ± 0.01
O. borealis	Stem bark	*n*-Hep	**10% ± 0.02**	19% ± 0.08
		DCM	**3% ± 0.02**	2% ± 0.02
		EtOAc	23% ± 0.07	nd[a]
		MeOH 80%	**7% ± 0.05**	0% ± 0.00
O. elatum	Stem bark	*n*-Hep	**5% ± 0.02**	47% ± 0.38
		DCM	**7% ± 0.03**	**57% ± 0.13**
		EtOAc	**11% ± 0.01**	13% ± 0.19
		MeOH 80%	31% ± 0.05	nd[a]
T. coronatum	whole plant	*n*-Hep	23% ± 0.05	nd[a]
		DCM	**15% ± 0.5**	**72% ± 0.36**
		EtOAc	**12% ± 0.03**	40% ± 0.31
		MeOH 80%	**7% ± 0.01**	3% ± 0.04

983 *In vitro* anti-*Toxoplasma* activity of extracts exhibiting less than 20% cytotoxicity at 25 µg/mL (N=6).

984 [a] nd=not determined

985 Positive *anti-toxoplasmic* activity control (pyrimethamine): 70-90% parasite growth inhibition rate at 1 µM.

986 Positive cytotoxicity control (pyrimethamine): 60-70% cytotoxicity at 20 µg/mL (i.e. 80 µM).

Table 3

Cytotoxicity and anti-*Toxoplasma* activities of fractions obtained from MeOH 80% extract of *E. guineensis* against *T. gondii* in Vero cells at 25 µg/mL (N=10).

Fractions from hydromethanolic extract of *E. guineensis*	Molecular family	Cytotoxic activity against Vero cells	Anti-*Toxoplasma gondii* activity
MeOH 80% extract	-	**5% ± 0.04**	**94% ± 0.07**
HP_{1A}	Saccharides	**7%± 0.09**	2% ± 0.02
HP_{1B}	Glycerolipids	**4% ± 0.08**	5% ± 0.07
HP_2	Flavonoids	**6% ± 0.07**	6% ± 0.10
HP_3	Flavonoids, terpenoids	**2% ± 0.04**	8% ± 0.12
HP_4	Flavonoids, terpenoids and sterols	**3% ± 0.02**	1% ± 0.02
HP_5	Terpenoids, flavonoids and fatty acids	67% ± 0.08	nd[a]
CPC_1	Fatty acids and glycerolipids	81% ± 0.07	nd[a]
CPC_2	Terpenoids and sterols	77% ± 0.04	nd[a]
CPC_3	Terpenoids and sterols	47% ± 0.07	nd[a]
CPC_4	Sesquiterpenoids	**9% ± 0.17**	22%± 0.015
CPC_5	Sesquiterpenoids	**8% ± 0.09**	8% ± 0.07
CPC_6	Saccharides	**5% ± 0.04**	0% ± 0.0
CPC_7	Saccharides	**4% ± 0.04**	15% ± 0.15
CPC_8	Flavonoids	**2% ± 0.04**	1% ± 0.01
CPC_9	Sesquiterpenoids and glycosides	**3% ± 0.02**	1% ± 0.01
CPC_{10}	Sesquiterpenoids and glycosides	**5% ± 0.10**	11% ± 0.13
CPC_{11}		**1% ± 0.01**	7% ± 0.07

[a] nd = not determined

Positive *anti-toxoplasmic* activity control (pyrimethamine): 70-90% parasite growth inhibition rate at 1 µM.

Positive cytotoxicity control (pyrimethamine): 60-70% cytotoxicity at 20 µg/mL (i.e. 80 µM).

Table 4

Cytotoxicity and anti-*Toxoplasma* activities of some fractions and combinations of MeOH 80% extract of *E. guineensis* (N=6).

Fractions	Concentration in µg/mL	Cytotoxicity against Vero cells	Anti-*Toxoplasma* activity at 25 µg/mL	Anti-*Toxoplasma* activity at 100 µg/mL
HP_{1A}	23.5	nd[a]	nd[a]	nd[a]
HP_5	1.5	0± 0.00	nd[a]	nd[a]
HP_{1A}+HP_5	25	**3% ± 0.01**	**0± 0.00**	**0± 0.00**
HP_{1B}	11.1	nd[a]	nd[a]	nd[a]
HP_5	13.9	40% ± 0.01	nd[a]	nd[a]
HP_{1B}+HP_5	25	**19% ± 0.01**	**64% ± 0.05**	**92% ± 0.02**
HP_2	14.2	nd[a]	nd[a]	nd[a]
HP_5	10.8	30% ± 0.01	nd[a]	nd[a]
HP_2+HP_5	25	**7% ± 0.05**	**67% ± 0.03**	**85% ± 0.03**
HP_3	19	nd[a]	nd[a]	nd[a]
HP_5	6	**15% ± 0.01**	1% ± 0.00	nd[a]
HP_3+HP_5	25	**2% ± 0.01**	**50% ± 0.08**	**83% ± 0.05**
HP_4	16.9	nd[a]	nd[a]	nd[a]
HP_5	8.1	20% ± 0.01	nd[a]	nd[a]
HP_4+HP_5	25	**10% ± 0.07**	**74% ± 0.02**	**92% ± 0.03**

[a] nd = not determined

Positive *anti-toxoplasmic* activity control (pyrimethamine): 70-90% parasite growth inhibition rate at 1 µM.

Positive cytotoxicity control (pyrimethamine): 60-70% cytotoxicity at 20 µg/mL (i.e. 80 µM).

1002 **Table 5**

1003 Plant species analyzed for their anti-*Toxoplasma* activity.

Botanical name (family)	Part used	Collection area	Collection date	Voucher no
Combretum micranthum G. Don (Combretaceae),	Leaf	Waninou, north-west Côte d'Ivoire GPS: 10.06763; 12.84672	16/02/2021	UCJ002988
Elaeis guineensis Jacq. (Arecaceae)	Leaf	Adiopodoumé, south Côte d'Ivoire GPS: 5.33003; 4.13064	29/02/2021	UCJ0141165
Erigeron floribundus (Kunth) Sch.Bip. (Asteraceae)	Aerial parts	Zagné, south-west Côte d'Ivoire GPS: 6.21500; 7.48823	25/02/2021	UCJ003610
Oldfieldia africana Benth. & Hook. f. (Picrodendraceae)	Stem bark	Zagné, south-west Côte d'Ivoire GPS: 6.21500, 7.48823	23/02/2021	UCJ006232
Octoknema borealis Hutch. & Dalziel (Olacaceae)	Leaf and stem bark	Azaguié, south-east Côte d'Ivoire GPS: 5.78954, 4.14758	07/02/2021	UCJ013216
Omphalocarpum ahia A. Chev. (Sapotaceae)	Leaf and stem bark	Keibly, south-west Côte d'Ivoire GPS: 5.99994, 7.7524	24/02/2021	WAG1830636
Omphalocarpum elatum Miers (Sapotaceae)	Stem bark	Azaguié, south-east Côte d'Ivoire GPS: 5.78954, 4.14758	07/02/2021	UCJ016568
Tristemma coronatum Benth. (Melastomataceae)	Whole parts	Yacassé-Mé, south-east Côte d'Ivoire GPS: 5.76860, 3.99075	07/02/2021	CSRS004223

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Highlights

- Antiparasitic activity of 40 different extracts from eight plants were investigated.
- Hydromethanolic extract of *Elaeis guineensis* leaves inhibited 94% parasite growth.
- Three nor-sesquiterpenes and one benzyl glycoside have been undescribed.
- Some binary combinations of fractions have recovered antitoxoplasmic activity.
- Nine compounds were identified by ^{13}C-NMR based dereplication.
- Fifteen compounds were purified from the two fractions.

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Declaration of interests

Anti-*Toxoplasma gondii* screening of eight species used in Côte d'Ivoire, and bio-guided isolation of three previously undescribed nor-sesquiterpenoids and one benzyl glycoside from *Elaeis guineensis* leaves

Nangouban Ouattara[a,b,d*], Mansour Issoufou-Tiny[a], Abdulmagid Alabdul Magid[a], Sandie Escotte-Binet[b,c], Philomène Akoua Yao-Kouassi[d,e], Isabelle Villena[b,c], Laurence Voutquenne-Nazabadioko[a].

[a] Université de Reims Champagne-Ardenne, CNRS, ICMR 7312, 51097, Reims, France.
[b] Université de Reims Champagne Ardenne, ESCAPE UR 7510, 51097, Reims, France.
[c] Centre National de Référence de la Toxoplasmose, CHU Reims, 51092 Reims, France.
[d] Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et de Technologie, Université Félix HOUPHOUET-BOIGNY 22 BP 582 Abidjan, Côte d'Ivoire.
[e] Université Polytechnique de San Pedro, BP V1800 San Pedro, Côte d'Ivoire.

*Corresponding authors
E-mail address: nangouban.ouattara@u-bordeaux.fr (Dr Nangouban OUATTARA)

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