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RESEARCH ARTICLE

Antiparasitic Activities of Plants From the Traditional Senegalese Pharmacopoeia: Isolation of Antitrypanosomal and Antileishmanial Ellagic Acid Derivatives From *Terminalia Avicennioides*

Abda Ba[1,2] | Vincent Roumy[1] | Kristelle Hughes[3] | Jennifer Samaillie[1] | Maude Bourlet[3] | Thierry Hennebelle[1] | Samba Fama Ndoye[2] | Marie-France Herent[3] | Joëlle Quetin-Leclercq[3] | Matar Seck[2] | Céline Rivière[1]

[1]Joint Research Unit 1158 BioEcoAgro, Univ. Lille, Junia, INRAE, Univ. Liège, UPJV, Univ. Artois, ULCO, Lille, France | [2]Laboratoire de Chimie Organique et Thérapeutique, Faculté de Médecine, de Pharmacie et d'Odontologie de l'Université Cheikh Anta Diop de Dakar, Dakar-Fann, Senegal | [3]Pharmacognosy Research Group, Louvain Drug Research Institute, Université Catholique de Louvain (UCLouvain), Brussels, Belgium

Correspondence: Céline Rivière (celine.riviere@univ-lille.fr)

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ABSTRACT

Parasitic diseases such as trypanosomiasis and leishmaniasis pose significant health challenges in Africa. The Senegalese Pharmacopoeia, known for its many medicinal plants with anti-infectious properties, can be a source of antiparasitic natural products. This study aimed to evaluate the in vitro antiparasitic activities of 33 methanolic extracts from 24 ethnopharmacologically selected plants against *Trypanosoma brucei brucei* (Tbb) and *Leishmania mexicana mexicana* (Lmm), as well as their cytotoxic activities on WI-38 cells. The most promising plant species were *Balanites aegyptiaca*, *Eucalyptus camaldulensis*, *Vachellia nilotica* and four Combretaceae species. In a second step, antitrypanosomal and antileishmanial compounds were isolated by preparative high-performance liquid chromatography from one of the most active plant extracts, *Terminalia avicennioides* leaves. Four ellagic acid derivatives were identified by nuclear magnetic resonance (1D & 2D) and high-resolution electrospray ionization-tandem mass spectrometry (HR-ESI-MS/MS) as 3,4,3-tri-*O*-methylellagic acid (**2**), 3,4,5-*O*-trimethyl-3',4'-*O*,*O*-methylideneflavellagic acid (**3**), hexamethylcoruleoellagic acid (**4**) and pentamethylflavellagic acid (**5**). In addition, HR-ESI-MS/MS-based dereplication analysis was performed to putatively identify compounds in active cyclohexane and dichloromethane sub-extracts. Pentamethylflavellagic acid (**5**) showed the most promising activity against Tbb (half-maximal inhibitory concentration = 6.20 µM). This is the first time that these compounds have been isolated from *T. avicennioides* and evaluated for their antitrypanosomal and antileishmanial activities.

1 | Introduction

Parasitic infections can cause many diseases in Africa. Among these, the human African trypanosomiasis (HAT), also known as "sleeping sickness", is a vector-borne parasitic disease caused by *Trypanosoma brucei* and is considered a neglected tropical disease (NTD) [1, 2]. This pathology, endemic in sub-Saharan Africa, particularly affects rural populations living from agriculture, livestock, fishing, or hunting [3, 4]. At least 54 million people have varying levels of risk for sleeping sickness [5]. HAT is highly lethal if left untreated and can lead to neurological and behavioural disorders [3]. Leishmaniasis, a parasitic infection caused by various

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Leishmania species, can result in severe clinical manifestations affecting the skin, mucous membranes, and visceral organs. In the advanced stage, it can lead to ulcerative destruction of the nose (such as perforation of the nasal septum), mouth, and pharynx with a fatal outcome without the introduction of treatment [6–8] Two million new cases per year are observed and 12 million people are currently infected. Visceral leishmaniasis (VL) is the most virulent with 500 000 annual cases and causing more than 50 000 deaths per year. This disease is prevalent in 88 countries and has a significant impact on West and East Africa [1, 9, 10].

Few drugs are available against these two infections (pentamidine, amphotericin B, suramin, miltefosine, melarsoprol, eflornithine, nifurtimox, etc.) due to several factors: numerous side effects, complex route of administration, need for qualified health personnel, cost, lack of supply in endemic areas [9, 11–13]. It is therefore necessary to find new antileishmanial and antitrypanosomal compounds that are effective and accessible to people in rural areas.

In Senegal, many plants are traditionally used for their antiparasitic properties, particularly in the treatment of malaria, trypanosomiasis and leishmaniasis. Among the most used plant families are the Combretaceae comprising several species such as *Guiera senegalensis* J.F.Gmel., *Combretum glutinosum* Perr. Ex DC., *Combretum micranthum* G. Don, *Terminalia leiocarpa* Baill., and *Terminalia avicennioides* Guill. & Perr. [14–16]. However, the anti-infectious properties of most of these plants have not yet been sufficiently scientifically verified.

In this study, 33 crude methanolic extracts from 24 plant species were evaluated for their antiparasitic properties against *Leishmania mexicana mexicana* (Lmm) and *T. brucei brucei* (Tbb), as well as for their cytotoxic activity against non-cancerous human lung fibroblasts WI-38. These plants had been previously selected on the basis of an ethnopharmacological survey carried out in Senegal for their anti-infectious properties [17]. A guided investigation was performed on *T. avicennioides*, one of the most active species, as part of this work to identify and isolate the compounds responsible for these antiparasitic activities.

2 | Results and Discussion

2.1 | Antiparasitic Activity of Crude Methanolic Extracts

In addition to our previous ethnopharmacological study [17] on the uses of Senegalese medicinal plants in the management of respiratory tract infections, the in-depth bibliographic study of the traditional Senegalese pharmacopoeia and the scientific literature showed that these plants could produce many potential antiparasitic metabolites. Concerning some plant species cited in our previous study [17], *G. senegalensis* J.F.Gmel. [18], *C. glutinosum* Perr. Ex DC. [19], *Vachellia nilotica* subsp. tomentosa (Benth.) Kyal. & Boatwr [20], *Eucalyptus camaldulensis* Dehnh. [21], *Senna occidentalis* (L.) Link [22], *T. avicennioides* Guill. & Perr. [23], *Strychnos spinosa* Lam [24] and *Spermacoce verticillata* L. [25, 26], only crude extracts or sub-extracts have been studied and no compound responsible for antitrypanosomal or antileishmanial activities has yet been isolated (Table S1).

First, the 33 methanolic crude extracts were tested on the two protozoa, Tbb and Lmm, at two concentrations (50 and 100 µg/mL) (Table 1). Cytotoxicity was also evaluated on non-cancerous human lung fibroblasts WI-38 to determine the selectivity index (SI). The findings revealed that the Tbb strain exhibited greater sensitivity to the extracts compared to the Lmm strain. Most of the extracts (24/33) showed interesting activities against the Tbb strain at 50 µg/mL (less than 40% parasite survival), while only *Balanites aegyptiaca* (0.9 ± 0.0%) and *E. camaldulensis* (1.0 ± 0.8%) were active against Lmm at the same concentration. However, twelve extracts strongly inhibited Lmm at 100 µg/mL (Table 1). Secondly, crude methanolic extracts active at both 50 µg/mL against Tbb and 100 µg/mL against Lmm were selected for the determination of IC_{50} against Tbb. Only the IC_{50s} of crude methanolic extracts of *B. aegyptiaca* and *E. camaldulensis* could be determined against Lmm (Table 2).

The results highlighted the strong antitrypanosomal activity of all selected plants belonging to the Combretaceae family associated with low cytotoxicity. Among them, *T. avicennioides* showed promising activities against Tbb: leaves (IC_{50} = 11.53 ± 3.25 µg/mL), root bark (IC_{50} = 16.67 ± 0.61 µg/mL), and roots (IC_{50} = 6.26 ± 0.18 µg/mL) with moderate to low cytotoxicity. In contrast, *G. senegalensis* (leaves) and *C. glutinosum* (leaves) extracts showed antiparasitic activities against Tbb (IC_{50} = 12.33 ± 2.45 and 17.29 ± 0.15 µg/mL respectively), but with lower selectivity values (Table 2). Some antiparasitic activities of these species have already been documented in the scientific literature. This is the case of the ethanolic extract of *C. glutinosum* leaves, active against *T. b. rhodesiense* (IC_{50} = 26.5 µg/mL) [27] and the dichloromethane extract of *G. senegalensis* leaves against *T. brucei* in vitro and in vivo in mice [18]. Antiplasmodial compounds were also isolated from *G. senegalensis*. Guieranone A showed the highest antimalarial activity against *P. falciparum* W2 strain (IC_{50} 1.29 µg/mL) followed by tetrahydroharman, harman and harmalan (IC_{50} 8.56, 3.29 and 22.43 µg/mL, respectively) [18, 28]. Among species belonging to other botanical families, the crude extract of *V. nilotica* pods was the most active against Tbb (IC_{50} = 3.58 ± 1.47 µg/mL) with moderate cytotoxicity against WI-38 cells (CC_{50} = 66.28 ± 0.08 µg/mL). *V. nilotica* is already known for its high content of condensed and hydrolyzable tannins and its antimicrobial properties including its antitrypanosomal activity [17, 20, 29, 30]. The crude methanolic extract of *V. nilotica* trunk bark has also been previously tested against the promastigote (IC_{50} = 19.5 µg/mL) and amastigote (IC_{50} = 77.52 µg/mL) stages of *L. donovani* [20]. Although crude extracts of *B. aegyptiaca* (trunk bark) and *E. camaldulensis* (leaves) were the most active against Lmm (IC_{50} = 20.01 ± 0.53 and 62.64 ± 6.73 µg/mL, respectively) among all the extracts tested, they were more active against Tbb (IC_{50} = 13.37 ± 4.36 and 12.09 ± 2.82 µg/mL, respectively). Moreover, the trunk bark extract of *B. aegyptiaca* was highly toxic on WI-38 cells (CC_{50} = 14.02 ± 3.30 µg/mL). For the leaves of *E. camaldulensis*, their antitrypanosomal and antileishmanial activities have also been previously described [21, 31].

Regarding *T. avicennioides*, only the antitrypanosomal activities of the methanolic root extract have been previously reported with an IC_{50} = 12.5 µg/mL against Tbb. The potential bioactive compounds were suggested to be punicalagin, ellagic acid, flavogallonic acid and terchebulin [23]. Ellagic acid and its derivatives, as well as ellagitannins, were the major compounds detected

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TABLE 1 | Antiparasitic activities of methanolic crude extracts of selected plant species against the two parasites, *Trypanosoma brucei brucei* (**Tbb**) and *Leishmania mexicana mexicana* (**Lmm**), as well as cytotoxic activity on non-cancerous human lung fibroblasts WI-38. Data are expressed relative to the negative control, dimethyl sulfoxide (DMSO), as mean ± SD ($n = 2$ or $n = 3$ in triplicates).

Methanolic crude extracts	Tbb (% viability ± SD)		Lmm (% viability ± SD)		WI-38 (% viability ± SD)	
	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
Balanites. aegyptiaca (L.) Delile (**Tb**)	0.8 ± 0.6	0.3 ± 0.0	0.9 ± 0.0	0.0 ± 0.0	1.3 ± 0.0	1.1 ± 0.5
Bauhinia rufescens Lam. (**L**)	3.4 ± 2.1	0.5 ± 0.3	117.7 ± 18.0	107.1 ± 8.5	87.4 ± 2.5	74.8 ± 0.4
Bauhinia Rufescens Lam. (**P**)	0.4 ± 0.3	0.6 ± 0.5	106.4 ± 7.9	81.9 ± 16.4	84.2 ± 2.9	78.3 ± 3.8
Bauhinia Rufescens Lam. (**Tb**)	2.0 ± 0.0	0.0 ± 0.0	112.8 ± 15.6	132.6 ± 39.1	84.5 ± 4.2	78.3 ± 2.6
Boscia Integrifolia J.St.-Hil. (**L**)	92.9 ± 3.5	68.7 ± 11.3	91.4 ± 4.2	86.6 ± 1.3	84.8 ± 0.6	87.4 ± 1.7
Chrozophora senegalensis (Lam.) A.Juss. ex Spreng. (**Wp**)	0.7 ± 0.7	0.1 ± 0.1	110.6 ± 12.3	95.0 ± 4.1	96.0 ± 1.4	99.6 ± 0.4
Cochlospermum tinctorium Perrier ex A. Rich. (**R**)	22.3 ± 3.2	0.1 ± 0.1	89.6 ± 2.2	112.8 ± 4.8	91.6 ± 0.6	70.2 ± 3.4
Combretum glutinosum Perr. Ex DC.(**L**)	0.2 ± 0.1	0.0 ± 0.0	104.6 ± 41.2	1.2 ± 0.1	98.1 ± 0.7	48.1 ± 2.9
Combretum micranthum G. Don (**R**)	24.7 ± 4.5	0.4 ± 0.3	45.8 ± 3.2	11.9 ± 1.7	91.6 ± 1.1	88.1 ± 9.8
Eucalyptus camaldulensis Dehnh. (**L**)	1.0 ± 0.6	0.2 ± 0.3	1.0 ± 0.8	1.0 ± 1.4	3.7 ± 0.1	2.0 ± 0.7
Ficus sur Forssk.(**R**)	15.3 ± 1.7	7.3 ± 1.4	97.2 ± 6.2	106.7 ± 0.6	88.0 ± 0.6	95.0 ± 2.3
Ficus sycomorus L. (**L**)	91.1 ± 2.7	1.2 ± 1.1	109.1 ± 18.3	108.1 ± 19.5	82.0 ± 0.5	80.4 ± 1.5
Ficus sycomorus L. (**Tb**)	0.6 ± 0.0	0.0 ± 0.0	104.1 ± 19.5	125.7 ± 33.0	89.0 ± 4.9	27.0 ± 0.5
Guiera senegalensis J.F.Gmel. (**L**)	1.4 ± 0.6	0.6 ± 0.3	49.2 ± 3.0	0.4 ± 0.3	84.2 ± 1.9	21.5 ± 0.3
Hibiscus sabdariffa L. (**C**)	89.8 ± 2.9	74.8 ± 3.0	101.4 ± 1.4	105.5 ± 3.0	81.9 ± 2.2	79.5 ± 4.0
Khaya senegalensis (Desv.) A.Juss. (**Tb**)	2.7 ± 2.7	0.6 ± 0.6	86.6 ± 1.1	64.0 ± 30.0	89.2 ± 0.0	78.2 ± 4.7
Mitragyna inermis (Willd.) Kuntze (**L**)	101.1 ± 14.6	0.6 ± 0.3	111.7 ± 13.3	99.0 ± 1.0	105.5 ± 5.0	78.6 ± 8.7
Piliostigma reticulatum (DC.) Hochst. (**L**)	57.8 ± 20.0	1.1 ± 0.1	107.8 ± 7.0	113.1 ± 12.9	98.0 ± 6.3	47.9 ± 4.9
Piliostigma reticulatum (DC.) Hochst. (**Tb**)	0.2 ± 0.0	0.0 ± 0.0	102.7 ± 7.3	133.7 ± 39.8	88.5 ± 1.4	9.2 ± 4.6
Pterocarpus erinaceus Poir. (**Tb**)	3.4 ± 2.0	14.2 ± 2.5	75.4 ± 7.1	76.2 ± 2.9	93.0 ± 0.8	11.9 ± 0.8
Senna italica Mill. (***L***)	28.7 ± 0.1	0.5 ± 0.0	68.2 ± 0.2	125.9 ± 0.2	106.9 ± 9.6	79.6 ± 11.6
Senna occidentalis (L.) Link (**L**)	99.5 ± 6.1	35.5 ± 8.1	124.7 ± 21.9	123.7 ± 23.6	79.0 ± 0.2	83.4 ± 0.4
Senna occidentalis (L.) Link (**P**)	82.1 ± 0.2	86.0 ± 3.3	120.1 ± 18.2	109.0 ± 8.8	93.9 ± 1.8	79.2 ± 13.5
Senna occidentalis (L.) Link (**S**)	60.3 ± 5.3	54.9 ± 0.1	115.9 ± 20.3	95.5 ± 0.0	101.7 ± 12.5	47.1 ± 5.5
Spermacoce verticillata L. (**L**)	0.7 ± 0.2	0.5 ± 0.4	116.1 ± 21.5	19.1 ± 13.9	85.3 ± 0.6	88.1 ± 0.5
Spermacoce verticillata L. (**S**)	3.2 ± 1.5	1.7 ± 1.2	102.7 ± 1.4	11.2 ± 8.5	84.9 ± 4.6	77.5 ± 4.9
Strychnos spinosa Lam. (**L**)	47.8 ± 16.6	1.1 ± 1.5	106.4 ± 9.8	96.5 ± 0.0	68.1 ± 7.3	68.6 ± 1.8
Terminalia avicennioides Guill. & Perr. (**L**)	0.3 ± 0.1	0.2 ± 0.0	80.5 ± 15.0	4.9 ± 1.5	80.5 ± 1.7	76.3 ± 27.9
Terminalia avicennioides Guill. & Perr. (**R**)	0.3 ± 0.1	0.1 ± 0.0	70.4 ± 26.8	3.0 ± 0.8	81.6 ± 0.5	6.3 ± 0.1
Terminalia avicennioides Guill. & Perr. (**Rb**)	0.4 ± 0.3	0.1 ± 0.0	44.8 ± 13.3	3.4 ± 3.1	75.7± 0.6	91.3 ± 10.2
Terminalia leiocarpa Baill. (**L**)	0.5 ± 0.5	0.0 ± 0.0	94.3 ± 2.1	8.0 ± 2.2	87.2 ± 1.5	86.6 ± 1.9
Vachellia nilotica subsp. *tomentosa* (Benth.) Kyal. & Boatwr (**P**)	0.3 ± 0.2	0.0 ± 0.0	39.8 ± 13.7	0.2 ± 0.1	97.2 ± 0.8	20.7 ± 5.7
Vachellia seyal (Delile) P.J.H.Hurter (**Tb**)	0.5 ± 0.7	0.2 ± 0.2	94.7 ± 3.9	71.2 ± 2.7	93.8 ± 2.8	43.2 ± 7.8

Plant parts: **L**: leaves; **R**: roots; **Rb**: root bark; **Tb**: trunk bark; **S**: stem; **Wp**: whole plant; **P**: pods; **C**: calyces.

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TABLE 2 | Antiparasitic activities (IC_{50} in μg/mL) of the most active plant crude extracts against the two parasites, *Trypanosoma brucei brucei* (**Tbb**) and *Leishmania mexicana mexicana* (**Lmm**), as well as cytotoxic activity (CC_{50} in μg/mL) on non-cancerous human lung fibroblasts WI-38. Data were expressed as mean ± SD ($n = 3$ in triplicates).

Botanical families	Methanolic crude extracts	WI-38 CC_{50} ± SD (μg/mL)	*Tbb* IC_{50} ± SD (μg/mL)	SI (Tbb)	*Lmm* IC_{50} ± SD (μg/mL)	SI (Lmm)
Combretaceae	*T. avicennioides* (**L**)	>100	11.53 ± 3.25	>8.6	—	—
	T. avicennioides (**Rb**)	>100	16.67 ± 0.61	>5.9	—	—
	T. avicennioides (**R**)	66.15 ± 0.75	6.26 ± 0.18	10.5	—	—
	G. senegalensis (**L**)	77.93 ± 3.12	12.33 ± 2.45	6.3	—	—
	C. glutinosum (**L**)	78.28 ± 6.87	17.29 ± 0.15	4.5	—	—
	T. leiocarpa (**L**)	>100	11.84 ± 3.77	>8.4	—	—
	C. micranthum (**R**)	>100	31.05 ± 7.73	>3.22	—	—
Rubiaceae	*S. verticillate* (**S**)	>100	34.25 ± 3.51	>2.91	—	—
	S. verticillata (**L**)	>100	25.66 ± 6.16	>3.89	—	—
Fabaceae	*V. nilotica* (**P**)	66.28 ± 0.08	3.58 ± 1.47	18.5	—	—
	B. rufescens (**L**)	>100	49.35 ± 3.41	>2.02	—	—
Zygophyllaceae	*B. aegyptiaca* (**Tb**)	14.02 ± 3.30	13.37 ± 4.36	1.04	20.01 ± 0.53	0.7
Myrtaceae	*E. camaldulensis* (**L**)	66.15 ± 1.71	12.09 ± 2.82	5.47	62.64 ± 6.73	1.05

Plant parts: **L**: leaves, **R**: roots, **Rb**: root bark, **Tb**: trunk bark, **S**: stem, **P**: pod; ***Tbb*** = *Trypanosoma brucei brucei*, ***Lmm*** = *Leishmania mexicana mexicana*, **WI-38** = non-cancerous human lung fibroblasts; **IC_{50} or CC_{50} of positive controls (in μg/mL)**: camptothecin (WI-38) 0.029 ± 0.001, suramin (Tbb) 0.055 ± 0.001, pentamidine (Lmm) 0.087 ± 0.008; **Selectivity indexes** were calculated by **$SI = CC_{50(WI-38)}/IC_{50(Tbb\ or\ Lmm)}$**; **(-)** IC_{50} not could be determined.

in *T. avicennioides* roots. Ellagic acid and hydrolysable tannins displayed trypanocidal activity in vitro with minimum inhibitory concentration (MIC) values ranging from 7.5 to 31.5 μg/mL (14–91 μM) [23]. These results were consistent with our study. However, the antitrypanosomal and antileishmanial activities of *T. avicennioides* leaves have not been described previously. The phytochemistry of this plant part has also not been explored before. Therefore, bioguided isolation of antiparasitic compounds from the crude methanolic extract *T. avicennioides* leaves was carried out in this study.

2.2 | Antiparasitic Activity of Sub-extracts and Isolated Compounds of *T. avicennioides* Leaves

From the crude methanolic extract of *T. avicennioides* leaves, liquid/liquid extraction was performed to obtain four sub-extracts (cyclohexane, dichloromethane [DCM], EtOAc and aqueous) evaluated for their antiparasitic properties. The EtOAc sub-extract of *T. avicennioides* leaves showed the highest antitrypanosomal activity ($IC_{50} = 5.85 \pm 0.31$ μg/mL) but no antileishmanial activity (Table 3). The analysis of the ethyl acetate sub-extract by ultra-high-performance liquid chromatography-ultraviolet-mass spectrometry (UHPLC-UV-MS) (Figure 1B, Figure S1 and Table S3) highlighted the presence of phenolic compounds such as ellagitannins, gallic acid, ellagic acid and its derivatives as the main compounds, as well as proanthocyanins and various flavonoids. These compounds were already known for their antitrypanosomal properties and some had already been identified in *Terminalia* species [4, 23, 32]. Ellagic acid (**1**), a reference standard, was tested and showed a high anti-trypanosomal activity against Tbb with an IC_{50} of 3.35 ± 1.27 μg/mL (11.08 μM) and with a SI of 6.82, consistent with previous data [33]. By contrast, ellagic acid did not show antiparasitic activity against Lmm. Ellagic acid was also previously reported for its antibacterial, antiparasitic and antimalarial activity in vitro and in vivo [34].

On the other hand, the cyclohexane and DCM sub-extracts characterized by moderate activities against Tbb (IC_{50} = 16.26 ± 0.77 and 13.56 ± 3.50 μg/mL, respectively) were also active against Lmm (IC_{50} = 19.53 ± 1.13 and 20.74 ± 0.05 μg/mL, respectively). The chromatographic profiles of the cyclohexane and DCM sub-extracts obtained by UHPLC-UV-MS (Figures 1C,D) exhibited a similar chemical composition, but the DCM sub-extract showed higher levels of the major compounds visible on the chromatogram. This may explain their very similar antiparasitic activities. The antitrypanosomal and antileishmanial activities of the DCM sub-extract (IC_{50} = 13.56 ± 3.50 and 20.74 ± 0.05 μg/mL, respectively) justified its fractionation by preparative high-performance liquid chromatography (HPLC) which led to the isolation of four ellagic acid derivatives (**2–5**), including three coruleoellagic and flavellagic acid derivatives: 3,4,3′-tri-*O*-methylellagic acid (**2**) [35, 36], 3,4,5-*O*-trimethyl-3',4'-*O*,*O*-methylideneflavellagic acid (**3**) [37], hexamethylcoruleoellagic acid (**4**) [38] and pentamethylflavellagic acid (**5**) [38, 39] (Figure 2). Their structures were identified by comparison of nuclear magnetic resonance (NMR) and mass spectrometry (MS) data with those reported in the literature. The apolar compounds **3–5** were isolated for the first time in this plant. These specialized metabolites are rather rare in the plant kingdom and compound **5** was the major peak of the DCM sub-extract visible on the chromatogram at 254 nm.

Compounds **2–5** were tested in vitro against both parasitic strains and WI-38 cells (Table 3) and showed significant activities against

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TABLE 3 | Antiparasitic activities (IC_{50} in μg/mL) of the crude methanolic extract and sub-extracts of *T. avicennioides* leaves as well as isolated compounds against the two parasites, *Leishmania mexicana mexicana* (**Lmm**) and *Trypanosoma brucei brucei* (**Tbb**), and cytotoxic activity (CC_{50} in μg/mL) on non-cancerous human lung fibroblasts WI-38. Data were expressed as mean ± SD ($n = 3$ in triplicates).

Crude extract/Sub-extracts/Compounds	IC_{50} ± SD in μg/mL (μM)		CC_{50} ± SD in μg/mL (μM)	SI	
	Lmm	Tbb	WI-38	(Lmm)	(Tbb)
MeOH	NA	11.53 ± 3.25	>100	—	>8.6
EtOAc	NA	5.85 ± 0.31	73.27 ± 1.15	—	12.5
Cyclohexane	19.53 ± 1.13	16.26 ± 0.77	58.27 ± 1.77	3.0	3.6
DCM	20.74 ± 0.05	13.56 ± 3.50	34.63 ± 13.31	1.6	2.5
1	NA	3.35 ± 1.27 (11.08)	22.81 ± 1.07 (75.53)	—	6.8
2	29.13 ± 4.33 (84.68)	15.39 ± 1.14 (44.73)	28.46 ± 5.00 (82.72)	0.9	1.8
3	54.21 ± 6.08 (145.72)	32.85 ± 5.72 (88.31)	10.92 ± 0.07 (29.35)	0.2	0.3
4	26.42 ± 2.77 (63.21)	20.77 ± 0.28 (49.68)	>50	>1.9	>2.4
5	56.71 ± 2.91 (146.15)	2.41 ± 0.04 (6.20)	19.71 ± 1.21 (50.80)	0.3	8.2
Pentamidine	0.08 ± 0.01 (0.24)				
Amphotericin B	0.09 ± 0.01 (0.10)				
Suramin		0.05 ± 0.01 (0.04)			
Camptothecin			0.03 ± 0.00 (0.07)		

NA: no activity at 100 μg/mL; **Selectivity indexes** (**SI**) were calculated by $SI = CC_{50(WI-38)}/IC_{50(Tbb\ or\ Lmm)}$.

Tbb with IC_{50} ranging from 2.41 to 32.85 μg/mL. Compound **5** was the most active against Tbb (IC_{50} = 2.41 ± 0.04 μg/mL either 6.20 μM) and had the best SI (= 8.2) regarding the CC_{50} obtained against WI-38 cells (CC_{50} = 19.71 ± 1.21 μg/mL either 50.80 μM) However, Lmm appeared to be more resistant to the isolated compounds with IC_{50s} ranging from 26.42 to 56.71 μg/mL. Hexamethylcoruleoellagic acid (**4**) was the most active against Lmm compared to other analogues of the series (IC_{50} = 26.42 ± 2.77 μg/mL or 63.21 μM) and was not toxic against WI-38 at 50 μg/mL. The evaluation of the cytotoxicity on WI-38 cells of the other compounds **2**, **3** and **5**, on the other hand, showed that they were potentially toxic (10.92 ± 0.07 μg/mL < CC_{50} < 28.46 ± 5.00 μg/mL). These activities were determined for the first time for compounds **3–5**. A structure-activity relationship (SAR) study provides evidence of the importance of the number of methoxy groups, their position in the structure and the type of substitution at the C-5 position [35]. Indeed, the presence of methylenedioxy at the C-3/C-4 position (compound **3**) or of a methoxy group in C-5 (compound **4**) decreased the activity against Tbb, compared to compound **5**. By contrast, the presence of a methoxy group at C-5, such as in compound **4,** appears to slightly increase the antileishmanial activities and attenuate the cytotoxicity.

Some of these ellagic acid derivatives have already been described for other biological activities. Compound **3** revealed cytotoxic activity against P-388 leukaemia cells [37], and has also been reported as an antibacterial agent [35], an aldose reductase inhibitor (RLAR), an antioxidant agent, a T-cell proliferation regulator [38] and a megakaryopoiesis inductor [36]. Compound **4**, belonging to the coruleoellagic acid group, is relatively unusual in the plant kingdom [37], and has previously revealed moderate antibacterial activity against *Pseudomonas aeruginosa* with a MIC of 64 μg/mL [38].

2.3 | Phytochemical Composition of *T. avicennioides*

To compare the different chemical profiles of *T. avicennioides* leaves, root, and root bark extracts and to putatively identify unpurified compounds, UHPLC-UV-MS or UHPLC-linear ion trap (UHPLC-LTQ) orbitrap XL tandem MS (MS/MS) analyses were performed. The data of the mass spectra *(m/z)*, absorbances (UV *λ*max) and retention times of the detected compounds were compared with those obtained from other *Terminalia* species such as *T. catappa* L., *T. brownie* Fresen, *T. chebula* Retz., *T. laxiflora* Engl., etc. [41–52]. The annotation process relied on several confidence levels described as follows: level 1 (unambiguously identified metabolite, confirmed by at least two parameters of an authentic standard, such as retention time and MS/MS spectrum, purified compounds in the case of our study), level 2 (putatively annotated metabolite), level 3 (putatively annotated metabolite class) and level 4 (unidentified metabolite but molecular formula assigned) [53].

The analyses showed that the ethyl acetate sub-extracts of roots and root bark were mainly composed of ellagitannins, gallic acid (retention time [RT] 0.98 min) and its derivatives, ellagic acid (RT 6.11 min) and its derivatives including ellagic acid xylopyranoside (RT 5.57 min) and ellagic acid xyloside (RT 5.85 min) (Figure S1 and Table S3). Ellagic acid was the predominant compound in roots (peak area 30.35%) and root bark (peak area 32.36%) at 254 nm. By contrast, the ethyl acetate sub-extract of *T. avicennioides* leaves, although containing numerous gallic acid and ellagic acid derivatives as well as ellagitannins, also appeared to be rich in flavonoids, mainly flavone-*C*-glycosides (Figure S1 and Table S3). However, ellagic acid and derivatives, and ellagitannins remained the main compounds in the ethyl acetate sub-extract of *T. avicennioides* leaves.

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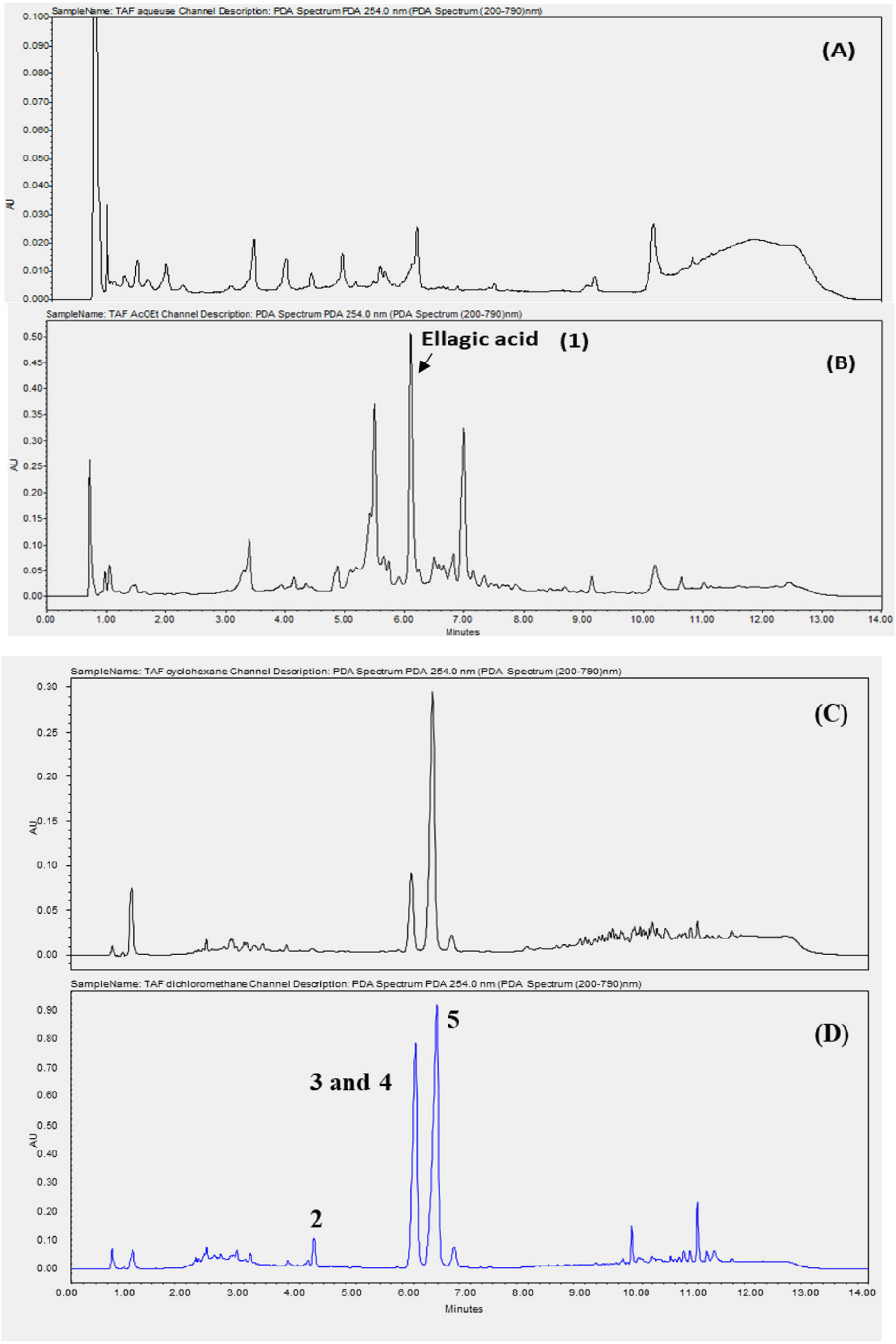


FIGURE 1 | Ultraviolet (UV) chromatograms obtained by ultra-high-performance liquid chromatography-UV-mass spectrometry (UHPLC-UV-MS) at $\lambda = 254$ nm of (A) Aqueous sub-extract, (B) EtOAc sub-extract, (C) Cyclohexane sub-extract and (D) Dichloromethane sub-extract of *T. avicennioides* leaves with purified compounds (**2**–**5**).

The analyses carried out on the cyclohexane and DCM sub-extracts of the leaves through UHPLC-UV-MS and UHPLC-LTQ orbitrap XL MS/MS (Figures S3 and S4) showed very similar chromatographic profiles with apolar ellagic acid derivatives (**2**–**5**) as major compounds (Figures 1C,D). In addition, other compounds such as orientin, vitexin, chlorophyll derivatives (epoxypheophorbide a, pheophorbide a, and their isomers), amides and alkaloids were detected in the cyclohexane and DCM sub-extracts of the leaves (Table 4 and Table S4). Pheophorbide a, known as a photosensitizer that is used in photodynamic therapy, induces photodynamic inactivation of *Trypanosoma cruzi* [54].

This dereplicative approach also revealed the detection of 3,4,3′-tri-*O*-methylellagic acid (RT 4.25 min) as a major compound of the dichloromethane root and root bark sub-extracts (Figure S2). However, the dichloromethane and cyclohexane sub-extracts from roots and root bark did not exhibit any antiparasitic activity against Tbb and Lmm, possibly suggesting a synergistic action of the ellagic acid apolar derivatives contained in the leaves.

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TABLE 4 | Identification of chemical constituents in cyclohexane and dichloromethane sub-extracts of *Terminalia avicennioides* leaves by a combination of ultra-high-performance liquid chromatography-photo diode array mass spectrometry (UHPLC-PDA-MS) and UHPLC-high-resolution tandem MS (UHPLC-HRMS/MS) data in positive ionization mode. Several levels of confidence were assigned (levels 1–3): Level 1 (unambiguously identified metabolite, purified compounds (**3–5**)), level 2 (putatively annotated metabolite matched with databases), level 3 (putatively annotated metabolite class, level not determined in this study) and level 4 (unidentified metabolite but molecular formula assigned).

RT (min)	λ_{max} (nm)	*m/z*	MS Major ion	MS/MS fragments [*m/z*]	Molecular formula	Δ ppm	Identification	Compound (sub)class	Confidence level	Database match information
3.63	263; 287 (sh); 346	449.1068	$[M + H]^+$	431.0970; 413.0872; 383.0759; 329.0659	$C_{21}H_{20}O_{11}$	−2.4	Orientin	Flavone-*C*-glycosides	2	KEGG C10114
3.71	279	433.1120	$[M + H]^+$	415.1014; 367.0807	$C_{21}H_{20}O_{10}$	−2.2	Vitexin	Flavone-*C*-glycosides	2	KEGG C01460
3.87		453.3424	$[M + H]^+$	435.3322	$C_{23}H_{48}O_8$	0.4			4	
4.27		340.2588	$[M + H]^+$	331.2539; 276.7275; 227.1750; 210.1486	$C_{18}H_{33}N_3O_3$	−2			4	
4.27		679.5097	$[M + H]^+$	661.5002; 643.4899; 452.3586; 435.3323	$C_{47}H_{66}O_3$	1.8			4	
4.27		163.1324	$[M + H]^+$	66.1679	$C_8H_{18}O_3$	−3			4	
5.09		453.3424	$[M + H]^+$	389.8113	$C_{23}H_{48}O_8$	0.4			4	
4.93	248; 329	227.1537	$[M + H]^+$	184.0990; 66.1679	$C_{15}H_{18}N_2$	−2.6	6-Methylergoline	Ergoline alkaloids	2	KEGG C07540
7.78		348.2741	$[M + H]^+$	266.0148; 188.9899	$C_{18}H_{37}NO_5$	−1.1			4	
8.30		181.1221	$[M + H]^+$	163.1114; 153.1270; 145.1009, 135.1165; 107.0852; 95.0852	$C_{11}H_{16}O_2$	−1.3	Dihydroactinidiolide	Benzofurans	2	PubChem CID 6432173
8.69		309.2060	$[M + H]^+$	291.1953	$C_{18}H_{28}O_4$	−0.2	5-O-Methylembelin	Phenolic lipids/Carbonyl compounds	2	KEGG C10373
8.87		295.1903	$[M + H]^+$	277.1796	$C_{17}H_{26}O_4$	−0.4	Phytuberin	Sesquiterpenoids	2	KEGG C09709
9.02	248; 303 (sh); 368	373.0552	$[M + H]^+$	358.0316; 343.0444; 327.0134; 312.0263	$C_{18}H_{12}O_9$	−0.6	3,4,5-*O*-Trimethyl-3',4'-*O*,*O*-methylideneflavellagic acid (**3**)	Hydrolyzable tannins	1	
9.04	248; 303 (sh); 368	419.0968	$[M + H]^+$	404.0732; 389.0498; 375.0708	$C_{20}H_{18}O_{10}$	−1.2	Hexamethylcoruleoellagic acid (**4**)	Hydrolyzable tannins	1	
9.10	247; 303 (sh); 370	389.0861	$[M + H]^+$	359.0399; 343.0449; 329.0656; 135.9100	$C_{19}H_{16}O_9$	−1.6	Pentamethylflavellagic acid (**5**)	Hydrolyzable tannins	1	
9.20		694.4000	$[M + H]^+$	679.9996; 604.1742; 372.9855; 195.3826	$C_{46}H_{51}N_3O_3$	−0.5			4	
9.26		288.2532	$[M + H]^+$	227.2002; 106.0859; 88.0753; 66.1679	$C_{16}H_{33}NO_3$	−0.5			4	
9.43		282.2062	$[M + H]^+$	124.0390; 66.1679	$C_{16}H_{27}NO_3$	−0.7			4	

(Continues)

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TABLE 4 | (Continued)

RT (min)	λ_{max} (nm)	*m/z*	MS Major ion	MS/MS fragments [*m/z*]	Molecular formula	Δ ppm	Identification	Compound (sub)class	Confidence level	Database match information
9.47		288.2533	$[M + H]^+$	270.2426; 227.2003; 106.0860; 88.0754; 66.1679	$C_{16}H_{33}NO_3$	−0.2			4	
9.57		557.2984	$[M + H]^+$	539.2882	$C_{30}H_{36}N_8O_3$	0.1			4	
9.69		532.3473	$[M + H]^+$	497.3103; 423.2742; 405.2633; 387.2523; 353.2685	$C_{27}H_{49}NO_9$	−1.4			4	
9.93		293.2111	$[M + H]^+$	275.2002	$C_{18}H_{28}O_3$	−0.2	(9S,13S,15Z)-12-Oxophyto-10,15-dienoate	Eicosanoids	2	KEGG C01226
10.05		304.2998	$[M + H]^+$	212.2370	$C_{21}H_{37}N$	−0.3			4	
10.22		295.2267	$[M + H]^+$	277.2160	$C_{18}H_{30}O_3$	−0.3	(9Z)-(13S)-12,13-Epoxyoctadeca-9,11-dienoic acid	Eicosanoids	2	KEGG C04594
10.29		414.2847	$[M + H]^+$	201.9759	$C_{22}H_{39}NO_6$	−0.8			4	
10.33	235; 328; 369; 434	810.5569	$[M + H]^+$	793.5292	$C_{41}H_{79}NO_{14}$	−0.6			4	
10.36	250; 274; 292	766.5303	$[M + H]^+$	749.5041; 705.4783; 335.2579; 291.2314	$C_{39}H_{75}NO_{13}$	−1.1			4	
10.38		722.5043	$[M + H]^+$	705.4775; 661.4504; 579.3372; 353.2164; 335.2578; 291.2320	$C_{37}H_{71}NO_{12}$	−0.9			4	
10.42		678.4779	$[M + H]^+$	661.4510; 617.4254; 573.3994; 535.3108; 335.2578; 309.1911; 291.2316	$C_{35}H_{67}NO_{11}$	−1.2			4	
10.44		634.4516	$[M + H]^+$	617.4249; 573.3990; 529.3734; 491.2846; 335.2579; 291.2316	$C_{33}H_{63}NO_{10}$	−1.4			4	
10.50	250; 298; 338	590.4254	$[M + H]^+$	573.3988; 529.3726; 485.3474; 447.2585; 335.2578; 291.2316	$C_{31}H_{59}NO_9$	−1.5			4	
10.52	229; 261; 271; 315	546.3993	$[M + H]^+$	529.3727; 403.2325; 335.2579; 291.2316	$C_{29}H_{55}NO_8$	−1.4			4	
10.55		502.3731	$[M + H]^+$	485.3464; 404.5136; 359.2061; 334.0398; 291.2323	$C_{27}H_{51}NO_7$	−1.5			4	
10.58		458.3471	$[M + H]^+$	441.3206; 315.1797	$C_{25}H_{47}NO_6$	−1.2			4	
11.11		609.2699	$[M + H]^+$	591.2590; 548.2731; 519.2379	$C_{35}H_{36}N_4O_6$	−1.5	Epoxypheophorbide a	Chlorins	2	KEGG C21192
10.87		332.3310	$[M + H]^+$	240.2682	$C_{23}H_{41}N$	−0.6			4	
10.91	252; 328; 345; 434	623.2491	$[M + H]^+$	605.2385; 573.2120; 545.2178	$C_{35}H_{34}N_4O_7$	−1.5			4	
11.09		607.2578	$[M + H]^+$	579.2589; 561.2497; 547.2692; 519.2382; 487.2120	$C_{35}H_{34}N_4O_6$	4.4			4	
11.31		326.3779	$[M + H]^+$	186.2213	$C_{22}H_{47}N$	−0.8			4	
11.73		593.2746	$[M + H]^+$	565.2805; 533.2543; 473.2336; 433.2389; 406.9892	$C_{35}H_{36}N_4O_5$	−1.8	Pheophorbide a	Chlorins	2	KEGG C18021
12.01		593.2748	$[M + H]^+$	565.2805; 533.2542; 473.2333; 406.9903	$C_{35}H_{36}N_4O_5$	−2.1	Pheophorbide a isomer I	Chlorins	2	
12.86		102.0337	$[M + H]^+$	90.5256; 61.0069	C_7H_3N	−1.5			4	

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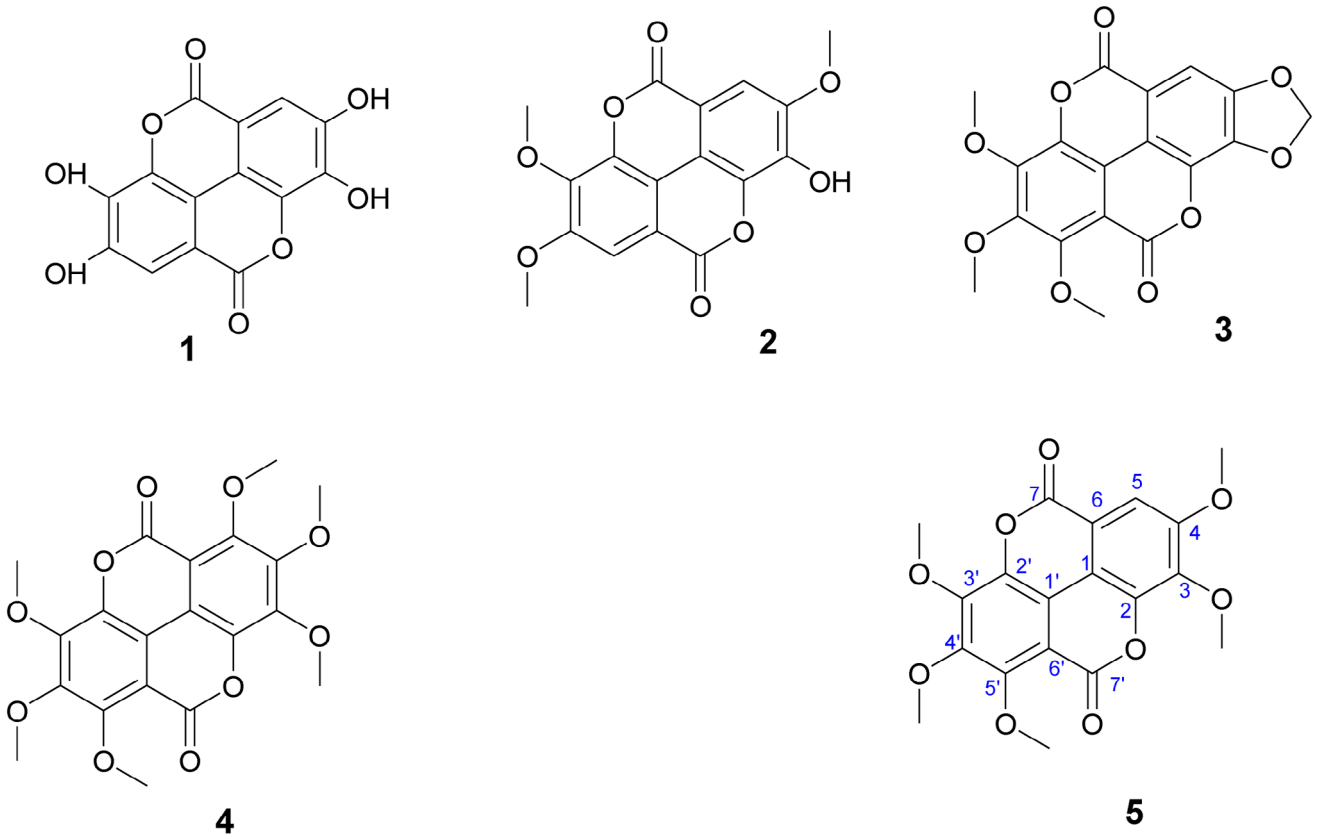


FIGURE 2 | Structures of ellagic acid (**1**); 3,4,3′-tri-O-methylellagic acid (**2**), 3,4,5-*O*-trimethyl-3',4'-*O*,*O*-methylideneflavellagic acid (**3**), hexamethylcoruleoellagic acid (**4**) and pentamethylflavellagic acid (**5**).

These phytochemical studies performed on *T. avicennioides* complemented the data on *Terminalia* species known for their high content of ellagitannins, gallic acid and ellagic acid derivatives as well as flavone-*C*-glycosides [50–52].

3 | Conclusions

The antiparasitic activities of the crude methanolic extracts of 24 selected plant species were evaluated in vitro on two parasites, Tbb and Lmm. In addition, the cytotoxic activity of the crude extracts was evaluated on non-cancerous human lung fibroblasts WI-38. The crude extracts of *T. avicennioides*, *C. glutinosum*, *G. senegalensis*, *C. micranthum*, *T. leiocarpa*, *V. nilotica*, *S. verticillata*, *Bauhinia rufescens*, *E. camaldulensis* and *B. aegyptiaca* showed the best antiparasitic activities. The results thus confirmed the antiparasitic properties of some plants traditionally used in Senegal, supporting their potential use as an alternative for the treatment of trypanosomiasis and leishmaniasis.

This study also specifically investigated the chemistry and antiparasitic activities of *T. avicennioides*, in particular leaf extracts. A dereplicative approach performed by UHPLC-LTQ orbitrap XL MS/MS described *T. avicennioides* as a source of hydrolyzable tannins, gallic and ellagic acid derivatives as well as flavone-*C*-glycosides. The good antitrypanosomal activity of *T. avicennioides* leaf extracts has been specifically highlighted. Bioguided purification led to the isolation of four apolar ellagic acid derivatives (**2–5**) including three coruleoellagic and flavellagic acids described for the first time in this species.

This study highlights the potential of *T. avicennioides* leaves as a promising remedy for trypanosomiasis. Indeed, ellagic derivatives **2–5** have been described as potential trypanocidal compounds, with low cytotoxicity for compound **4**. Pentamethylflavellagic acid (**5**) showed the most promising antiparasitic activity against Tbb ($IC_{50} = 2.41 \pm 0.04$ μg/mL or 6.20 μM). In addition, compound **4** showed moderate antileishmanial activity ($IC_{50} = 26.42 \pm 2.77$ μg/mL or 63.21 μM). These compounds and their analogues should be considered for further structure-activity relationship studies and optimization of the antiprotozoal activity.

4 | Experimental

4.1 | General Experimental Procedures

LC-high resolution-electrospray ionization-tandem MS (LC-HR-ESI-MS/MS) analyses were performed on an Accela UHPLC system from Fisher Scientific (Thermo Fisher Scientific, Bremen, Germany) combined with a photodiode array detector (PDA) and a Thermo Fisher Scientific LTQ orbitrap XL mass spectrometer with ESI source. The LC system was piloted by ChromQuest software, while the mass spectrometry system was controlled by Xcalibur software. Data acquisition was performed under full scan switch positive mode ionization from *m/z* 100 to 2000 at

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a resolution of 30 000. UHPLC-UV-MS analyses were carried out on Waters ACQUITY equipment (Waters Acquity UPLC H-Class, Guyancourt, France) consisting of a UV photodiode array detector (PDA) and a quadrupole (QDA) mass spectrometer with ESI as ionization source in positive and negative mode from *m/z* 100 to 950. Preparative HPLC was performed on a Shimadzu chain equipped with an SPD-M20A UV diode array detector and a CBM-20A control system. 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC and HMBC) NMR spectra were obtained on a Bruker 500 MHz spectrometer (NEO AVANCE console), operating at 500 MHz for proton (^{1}H) and 125.77 MHz for carbon-13 (^{13}C). All spectra were acquired in deuterated chloroform ($CDCl_3$, 99.96% D, D029T; EurisoTop, France). Data were processed with Bruker TopSpin software (4.1.4). Extraction solvents (methanol, cyclohexane, dichloromethane, and ethyl acetate), acetonitrile and formic acid for LC-MS analysis were purchased from Carlo Erba Reagents (Val de Reuil, France). Ultrapure water was obtained after filtration on a system Millipore Integral 5 Milli-Q (Merck, Germany). The standard ellagic acid was previously isolated in the laboratory from the roots of *T. avicennioides* by CPC. The protocol was described in the Supporting Information.

4.2 | Plant Material

The plants studied were collected in Senegal during an ethnobotanical survey [17]. The 24 plant species collected were authenticated by the botanists Drs William Diatta and Mamadou Sidybé. Voucher specimens were deposited at the Dakar Herbarium (Department of Vegetable Biology of the Faculty of Science and Technology of the Cheikh Anta Diop University). Voucher specimen numbers, collection dates and GPS coordinates were specified in Table S2. For some species, different plant parts were studied. An overview of the chemical composition and biological activities of harvested plants is presented in Table S1.

4.3 | Plant Extraction

Plant parts of the selected botanical species were oven-dried at temperatures between 25°C and 37°C for two weeks in the dark and pulverized using a mechanical grinder (SK100; RETSCH, Germany).

Plant powders (20 or 100 g), stored in the dark and at room temperature, were subjected to a solid-liquid (S/L) extraction in methanol, with a ratio of 1/10. Three 24-h maceration sessions were performed using an orbital shaker (GFL 3015, Germany). At the end of each maceration, the filtered methanolic extract was evaporated at 35°C under vacuum by a rotary evaporator (Grosseron, Heidolph, Germany) to obtain crude extracts [17].

Following the results of the first biological screening, certain selected crude methanolic extracts were suspended in water (500 mL) and partitioned by successive liquid-liquid extractions with an equivalent volume of organic solvents of increasing polarity: cyclohexane, dichloromethane and ethyl acetate (Carlo Erba Reagents, Val de Reuil, France). The organic phases were dried with anhydrous sodium sulphate (Na_2SO_4), filtered and then evaporated under vacuum [17].

4.4 | Purification of *T. Avicennioides* Metabolites by Preparative HPLC

Preparative HPLC was performed on a Shimadzu system equipped with two LC-20AP pumps, a 500 µL injection loop, a Shimadzu SPD-M20A diode array UV detector and a CBM-20A control system. The column was an Interchim C18-HQ column (5 µm, 250 × 21.2 mm, Interchim, France) preceded by a C18-HQ pre-column (7.5 × 21.2 mm). The mobile phase consisted of two solvents (A) ultrapure water + 0.1% formic acid and (B) acetonitrile. Several injections of 500 µL of a stock solution of the DCM sub-extract of *T. avicennioides* leaves at 80 mg/mL in MeOH were performed. The flow rate was set at 16 mL/min. Preparative HPLC was performed following the elution program: 40% B (0–5 min), 40%–45% B (5–35 min), 45%–100% B (35–37 min) and 100% B (37–45 min). Fractionation monitoring was carried out at 2 main wavelengths: 254 nm and 280 nm. The pure compounds **2** (3.2 mg), **3** (6.9 mg), **4** (7.4 mg) and **5** (8.6 mg) were collected and dried. Their purity was evaluated by UHPLC-UV (Figures S5–S9).

4.5 | UHPLC-UV-MS Analysis

Extraction and purification were followed by UHPLC-UV-MS (Waters Acquity UPLC H-Class, Guyancourt, France). The system is equipped with two independent pumps, an automatic injector, a controller, a UV PDA and a QDA mass spectrometer with ESI as an ionization source. The stationary phase was an Interchim Uptisphere C18-AQ column (2.1 × 100 mm, 2.6 µm, Interchim, France) connected to a 0.2 µm in-line filter. The mobile phase was composed of two solvents: (A) ultrapure water + 0.1% formic acid (Carlo Erba Reagents, Val de Reuil, France) and (B) acetonitrile (Carlo Erba Reagents, Val de Reuil, France) + 0.1% formic acid. Different UHPLC-UV-MS methods were developed depending on the sample. The method suitable for the analysis of crude methanolic extracts, as well as EtOAc and aqueous sub-extracts was developed with the following elution gradient: 10% B (0–0.5 min), 10%–25% B (0.5–8.5 min), 25%–100% B (8.5–9 min), 100% B (9-11 min), before re-equilibration for 3 minutes. The analysis of cyclohexane and DCM sub-extracts, as well as their fractions was performed using another elution method: 10%–40% B (0–1 min), 40% B (1–6 min), 40%–100% B (6–9 min), and 100% B (9–11 min), before re-equilibration for 3 minutes. The flow rate was 0.3 mL/min. The temperature of the column was stabilized at 30°C. The UV detection was carried out between 200 and 790 nm. For mass spectrometry detection, the carrier gas was nitrogen. The voltage cone was 15 V with an ionization energy of 15 eV. Mass spectra were obtained in positive and negative mode in a *m/z* range of 100–950 uma, using Empower software.

4.6 | UHPLC-LTQ Orbitrap XL MS/MS Analysis for Dereplicative Approach

For cyclohexane and DCM sub-extracts of *T. avicennioides* leaves, further analyses were performed using a Fisher Scientific Accela UHPLC system from (Thermo Fisher Scientific, Bremen, Germany) driven by ChromQuest software consisting of a PDA detector, an autosampler, a tray compartment cooler (set to 20°C), a quaternary pump, and coupled to a Thermo Fisher Scientific LTQ orbitrap XL mass spectrometer. The same Interchim column

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and methods described above were used for UPLC-LTQ orbitrap XL MS analyses. HR-MS were measured with an ESI source in positive ionization mode using full-scan MS with a mass range of 100–2000 *m/z*. The orbitrap was operated at 30 000 resolutions (FWHM definition). All experimental data were acquired using a daily external calibration prior to data acquisition. Appropriate tuning of the electrospray ion source was performed, and the following electrospray inlet conditions were applied: flow rate: 1000 µL/min with a split of 50/50 before reaching the mass detector; spray voltage: 5 kV; sheath gas (N_2) flow rate: 20 a.u.; auxiliary gas (N_2) flow rate: 20 a.u.; discharge current of 5 µA; capillary temperature of 250°C; capillary voltage of 21 V and tube lens voltage of 75 V. For extracts, the data-dependent MS/MS events were performed on the most intense ion detected in full scans MS with a collision energy of 35 eV. Data acquisition and processing were performed with Xcalibur software.

4.7 | MZmine Data Preprocessing Analysis and Dereplication

Raw mass data was acquired by UPLC-LTQ orbitrap XL MS and converted to .mzML file format by using MSConvert software. All HRMS run data files (.mzML) were exported to the open-source software package MZmine 3 (v3.3.0) for data processing. Mass detection was realized with all exact mass detectors with noise level set to 1.0E4 for MS^1 level. For MS^2 detection, the noise level was set to 0. The ADAP Chromatogram builder was achieved using a minimum group size of scans of five, a minimum group intensity threshold of 1.0E4, a minimum highest intensity of 1.0E4, and a *m/z* tolerance of 0.01 (or 10 ppm). The smoothing parameters were set to 5. The ADAP wavelet was used for chromatogram deconvolution with the following settings: S/N threshold of 10, minimum feature height of 1.0E4, coefficient/area threshold of 10, peak duration range of 0.01–0.7 min, RT wavelet range of 0.01–0.10 min. The *m/z* and RT ranges for MS/MS scan pairing were set to 0.02 Da and 0.2 min, respectively. Isotopes were detected using the isotope peak grouper with a *m/z* tolerance of 10 ppm, an RT tolerance of 0.3 min (absolute), the maximum charge set at 1, and the representative isotope used was the most intense. Alignment was achieved with a *m/z* tolerance of 10 ppm and an RT tolerance of 0.7 min. Then, the aligned peak list was gap-filled with an RT range of 0.3 min and *m/z* tolerance of 10 ppm. The resulting list was filtered using the peak list row filter option to remove all the duplicates and features without the associated MS^2 spectrum. The prediction of the molecular formula of ions in positive mode was performed with MZmine with an *m/z* isotope tolerance of 5 ppm and a minimum score of 0.7. Putative identification of peaks was conducted by comparing MS/MS fragmentation patterns available in the literature and using the databases integrated into the MZmine software such as KEGG and PubChem.

4.8 | Structure Elucidation

The structures of the purified compounds were determined using HR-MS/MS, UV, and NMR data. The NMR mono-(^{1}H and ^{13}C) and two-dimensional (COSY, HSQC-DEPT, HMBC) in $CDCl_3$ were obtained with a 500 MHz Bruker spectrometer, NEO AVANCE console, operating at 500 MHz for proton (^{1}H) and 125 MHz for carbon-13 (^{13}C). Measurements were performed with a 5 mm TXI probe at 25°C. The NMR data were processed with Bruker TopSpin software (4.1.4).

Compound 3 (3,4,5-*O*-trimethyl-3',4'-*O*,*O*-methylideneflavellagic acid). White amorphous powder; HR-MS: *m/z* 373,0551 $[M + H]^+$, calcd. for $C_{18}H_{12}O_9$, 372.0484. MS/MS (75 eV, ESl): *m/z* 373 ($[M + H]^+$, 91%), 358 (100), 343 (19), 327 (3), 185 (0.3), 104 (0.3), 92 (0.3). UV spectrum: 248, 303 (sh), and 367 nm. Spectra ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H (ppm) 7.63 (1H, *s*, **H**-5), 6.31 (2H, *s*, —OC**H$_2$**O—), 4.29 (3H, *s*, 3'—OC**H$_3$**), 4.09 (3H, *s*, 5'—OC**H$_3$**), 4.03 (3H, *s*, 4'-OC**H$_3$**); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ_C (ppm) 158.4 (C-7), 154.7 (C-7′), 154.1 (C-5′), 151.1 (C-3'), 148.4 (C-3), 147.4 (C-4), 138.6 (C-4'), 137.8 (C-2'), 132.3 (C-2), 128.8 (C-6), 115.5 (C-1′), 114.4 (C-1), 112.8 (C-6'), 104.8 (C-5), 103.9 (3—OCH_2O), 62.3 (5′—OCH_3), 62.0 (3′—OCH_3), 60.4 (4′—OCH_3) (Figures S10 and S11).

Compound 4 (hexamethylcoruleoellagic acid). White amorphous powder; HR-MS: *m/z* 419.0966 $[M + H]^+$, calcd. for $C_{20}H_{18}O_{10}$, 418.0900. MS/MS (75 eV, ESl) *m/z* 419 ($[M + H]^+$, 57%), 404 (100), 389 (9), 375 (5). UV spectrum: 248, 303 (sh), and 370 nm. Spectra ^{1}H-NMR ($CDCl_3$, 500 MHz): all the protons appeared as three singlets at 4.26, 4.03, and 4.02 ppm and integrated for each 6 protons. ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 158.4 (2C), 154.7 (1C), 154.1 (1C), 151.0 (2C), 138.5 (4C), 112.8 (2C), 105.8 (2C), 62.4 (2C), 62.0 (4C) (Figures S12 and S13).

Compound 5 (pentamethylflavellagic acid). White amorphous powder; HR-MS: *m/z* 389,0871 $[M + H]^+$, calcd. for $C_{19}H_{16}O_9$, 388.0794. MS/MS, (75 eV, ESl) *m/z* 389 ($[M + H]^+$, 59%), 374 (100), 358 (2), 343 (1), 328 (1). UV spectrum: 247, 303 (sh), and 370 nm. Spectra ^{1}H ($CDCl_3$, 500 MHz): δ_H (ppm) 7.71 (1H, *s*, **H**-5'), 4.30 (3H, *s*, 3'—OC**H$_3$**), 4.24 (3H, *s*, 3—OC**H$_3$**), 4.07 (3H, *s*, 5'—OC**H$_3$**), 4.06 (3H, *s*, 4'—OC**H$_3$**), 4.03 (3H, *s*, 4—OC**H$_3$**). ^{13}C-NMR (125 MHz) δ_C (ppm) 158.8 (C-7), 155.4 (C-7′), 154.7 (C-5′), 153.8 (C-3'), 148.2 (C-4'), 147.3 (C-3), 141.6 (C-4), 137.5 (C-2′), 130.9 (C-2), 114.6 (C-1′), 113.1 (C-1), 112.9 (C-6), 107.4 (C-5), 105.7 (C-6'), 62.3 (5′—OCH_3), 62.1 (3′—OCH_3), 62.0 (3—OCH_3), 61.9 (4′—OCH_3), 56.7 (4—OCH_3) (Figures S14 and S15).

4.9 | Antiparasitic Activity Against Tbb and Lmm

The antiparasitic activity of plant extracts and pure compounds was evaluated in vitro on Tbb (strain 427) and on promastigotes of Lmm (MHOM/BZ/84/BEL46) according to the protocol described by Bero et al. [4] Extracts and pure compounds were solubilized initially in DMSO to obtain stock solutions of 20 mg/mL.

Tbb strains were cultured in HMI9 medium (Sigma-Aldrich) containing 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich, Germany), 150 mM L-cysteine and 20 mM beta-mercaptoethanol (Sigma-Aldrich, Germany) and were incubated at 37°C in an atmosphere moistened with 5% CO_2. Lmm strains were cultured in HOMEM medium (Sigma-Aldrich) with 15% heat-inactivated FBS and 5 µg/mL of hemin. Lmm strains were then incubated at 28°C in an atmosphere moistened with 5% CO_2. Suramin and pentamidine (Sigma-Aldrich, Germany) (2 mg/mL) were used as positive controls for antitrypanosomal and antileishmanial activities, respectively.

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All plant extracts were first evaluated at 50 and 100 µg/mL. Then the IC_{50} (concentration of extract capable of inhibiting 50% of the growth of parasites) were determined for the most active extracts and for the pure compounds. In this case, the extracts and purified compounds were serially diluted for eight concentrations from 100 to 0.046 µg/mL (or from 10 to 0.0046 µg/mL for positive controls). All tests were performed in a 96-well microplate containing 5.10^4 parasites/well. After 72 hours of incubation, 10 µL of an Alamar Blue solution diluted in PBS solution (1:1) were added. After 4 h of incubation, fluorescence at 590 nm was measured with a spectrophotometer (SpectraMax M2^e; Molecular Devices, UK). The tests were performed in triplicate and repeated three times. Statistical analyses were conducted using Microsoft Excel software or GraphPad Prism, version 8.4.2 (GraphPad Software, San Diego, CA, USA).

4.10 | Cytotoxic Activity against Human Lung Fibroblast Cell Line WI-38

The cytotoxic activity of the extracts and pure compounds was evaluated according to the protocol described by Bero et al. [55] on the non-cancerous human lung fibroblast cell line WI-38 (ATCC CCL-75, bought from LGC standards, Molsheim, France), using the MTT tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colourimetric method) [56].

All extracts and pure compounds were solubilized in DMSO to obtain a stock solution of 20 mg/mL. Camptothecin (2 mg/mL) (Sigma-Aldrich, Germany) was used as a positive control. All plant extracts were first tested at 50 and 100 µg/mL, then CC_{50} (cytotoxic concentration 50%) were determined for plants characterized by good antiparasitic activity and for pure compounds to determine the SI.

To determine the CC_{50}, cells were seeded in a 96-well microplate at a final concentration of 5000 cells/well in Dulbecco's Modified Eagle medium (DMEM) (Gibco) containing 4 mM L-glutamine, 1 mM sodium pyruvate and supplemented with 10% heat-inactivated FBS (Thermo Fisher Scientific, France) and antibiotics (penicillin/streptomycin mixture 100 UI/mL, Lonza, Verviers, Belgium). After 24 h incubation, serial dilutions of extracts or pure compounds in DMEM culture medium were added to obtain eight concentrations ranging from 100 to 0.046 µg/mL or from 10 to 0.0046 µg/mL (for the positive control). After 72 hours of incubation at 37°C in an atmosphere humidified with 5% CO_2, the culture medium was completely replaced with 100 µL MTT (Thermo Fisher Scientific, Illkirch, France), then the plates were incubated for 40 minutes. The MTT solution was replaced by 100 µL of DMSO (Sigma-Aldrich, Germany) and absorbance was measured at 570 and 620 nm using a spectrophotometer (SpectraMax M2^e, Molecular Devices, UK). The tests were performed in triplicate and repeated three times. The CC_{50} were determined using Microsoft Excel and GraphPad Prism software, version 8.4.2 (GraphPad Software, San Diego, CA, USA).

4.11 | Statistical Analysis

Data were analysed and processed using Microsoft Excel and GraphPad Prism 8.4.2 and by comparing each treated group and untreated group (DMSO control). Data were analysed by one-way ANOVA and were expressed as mean ± SEM from three independent experiments, performed in triplicates.

Author Contributions

Abda Ba: conceptualization, methodology, investigation, formal analysis, resources, funding acquisition and writing–original draft and editing. **Vincent Roumy**: supervision and writing–review and editing. **Kristelle Hughes**: methodology, formal analysis and writing–review and editing. **Jennifer Samaillie**: methodology, **Maude Bourlet**: methodology and resources. **Thierry Hennebelle**: visualization. **Samba Fama Ndoye**: visualization. **Marie-France Herent**: methodology, resources. **Joëlle Quetin-Leclercq**: methodology, resources, validation, data curation and writing–review and editing. **Matar Seck**: supervision, conceptualization, writing–review and editing, funding acquisition and project administration. **Céline Rivière**: supervision, conceptualization, methodology, validation, writing–review and editing, funding acquisition and project administration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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