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Traditional uses and pharmacological activities of *Tetracera alnifolia* wild

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ABSTRACT

Ethnopharmacological relevance: Tetracera alnifolia Wild, is well used in traditional Guinean medicine for the treatment of infectious skin diseases. The present aim was to contribute to the valorization of Tetracera alnifolia leaves, focused on ethnomedical, biological and phytochemical investigations.
Materials and methods: We conducted an ethnomedical survey across several markets of the city of Conakry to identify 39 healers. Chloroform, methanol, dichloromethane, and aqueous extracts were tested for activities against protozoa, bacteria, fungi, HIV, and SARS-CoV-2.
Results: The traditional healers indicated that T. alnifolia is used in the treatment of >15 pathologies including Fassa (marasmus/malnutrition), Soukhou kouyé (white discharge in women), and Tèmou bankhi (sexual weakness in men). Leaves were the most used part. The modes of preparation included decoction and powder. Data from biological activities identicatied good activities of the methanolic extract against Leishmania infantum (MIC = 8.11 μg / ml) and a moderate activity on Trypanosoma brucei (MIC = 28.15 μg / ml) and Staphylococcus aureus (MIC = 29.91 μg / ml), while dichloromethane extracts acted on live SARS-CoV-2 replication with up to 53.4 % inhibition at 50 μg/mL.
Conclusion: These results explain at least in part the traditional use of T. alnifolia.

1. Introduction

Before the development of antibiotics and vaccines, infectious and parasitic diseases were the leading cause of death worldwide. They are now responsible for about 8 % of deaths in developed countries but up to 50 % of deaths in low-income countries (Murray et al., 2022). Within the context of increased poverty and malnutrition, combined with limited hygiene availability and access to high education, infectious and protozoan diseases affect millions of people every year in low-income countries and currently represent the primary cause of mortality in the tropical zone (Santos et al., 2020).

In Guinea, according to data from the Ministry of Health, eight neglected tropical diseases (onchocerciasis, lymphatic filariasis, trachoma, schistosomiasis, soil-transmitted helminths, leprosy, human African trypanosomiasis and Buruli ulcer), in addition to malaria, were considered a public health problem (Cherif et al., 2023). Also, >50 % of the population lives in highly rural areas where access to conventional healthcare facilities is rare. Taking into account these conditions, rural people in Guinea rely strongly on traditional herbal medicine to manage their healthcare, including to treat infectious diseases. (Baldé et al., 2006; Baldé et al., 2015; Diallo et al., 2012; Diallo et al., 2019; Magassouba et al., 2007; Traore et al., 2013).

Species from the *Tetracera* genus are commonly used for the treatment of various diseases including infections in many parts in Africa (Manuel et al., 2020; Roheem et al., 2020). *Tetracera alnifolia* Willd is a perennial, evergreen big liana of the family Dilleniaceae which commonly grows in the forests and other warm regions of sub-Saharan Africa including Guinea. Several parts of *T. alnifolia* widely available in local markets, have been traditionally used for treating infectious

Abbreviations: ATCC, American Type Culture Collection; BEI Resources, Biodefense and Emerging Infections Research Resources Repository; CFU, Colony-Forming Unit; HIV, Humanlimmunodeficiency Virus; IRDPMAG, Institut de Recherche et de Developpement des Plantes Médicinales et Alimentaires de Guinée; MIC, Minimum Inhibitory C oncentration; NIAID, National Institute of Allergy and Infectious Diseases; NIH, National Institutes of Health; NBT, nitroblue tetrazolium; PES, Phenazine Ethosulfate Solution; RPMI 1640, Roswell Park Memorial Institute medium; SDA, Sabouraud Dextrose Agar; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; $TaCHCl_3$, *Tetracera alnifolia* chloroformic extract; $TaCH_2Cl_2$, *Tetracera alnifolia* dichloromethanic extract; $TaCH_3OH$, *Tetracera alnifolia* methanolic extract; TaH2O, *Tetracera alnifolia* aqueous extract; TCID50, Median Tissue Culture Infectious Dose; TSB, Tryptic Soy Broth; WHO, World Health Organization

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diseases (including sexual transmitted disease), skin diseases, and malaria in Guinean traditional medicine. This study is part of the program of the Institute for Research and Development of Medicinal and Food Plants of Guinea (IRDPMAG), whose goal is to rationalize the integration of phytotherapy into our health systems as recommended by the WHO. In this context, based on the ethnomedical investigations conducted by IRDPMAG, *Tetracera alnifolia* was selected to confirm some of its traditional uses in Guinea as well as to survey its chemical and biological properties

2. Materials and methods

2.1. Ethnomedical survey

Our survey took place across several markets in Conakry, Guinea including Niger, Madina, Bonfi, Gbessia, Koloma, Enco 5, Taouyah, Kenien and Matoto markets. We opted for individual contact with healers in these markets. For consenting healers, we used the interactive method of interviewing the healers about their knowledge in the form of a semi-structured interviews following the outline of the survey questionnaire. Each questionnaire included demographic information such as sex, age, status but also description of diseases according to the healers, the mode of acquisition of the knowledge, and the modes of preparation and administration of the recipes. The study protocol was approuved by the IRDPMAG ethics committee.

2.2. Plant material

T alnifolia material was collected in the prefecture of Dubréka (Guinea). Leaves were dried at room temperature. The vouchers specimens were deposited in the herbarium of IRDPMAG (Voucher reference: 44HK470) and in the Guinean National Herbarium (Voucher reference D42HK2).

2.3. Preparation of plant extracts

T alnifolia material was air dried and then ground in a mill. Powders (10 g) were submitted to maceration for 24 h with 100 mL chloroform, 100 mL methanol and 150 mL of distilled water, separately. The chloroform and methanol extracts were filtered, pooled, evaporated *in vacuo* at < 40 °C in a rotary evaporator, coded ($TaCHCl_3$; 20 mg) ($TaCH_3OH$; 40 mg) respectively and stored at −80 °C until tested. The filtered aqueous extract was lyophilized to give aqueous extract (TaH_2O; 50 mg).

For SARS-CoV-2 test a part of aqueous extract (20 mg) was dissolved in water and treated by dichloromethane (100 mL). The dichloromethane fractions were filtered, pooled, evaporated *in vacuo* at < 40 °C in a rotary evaporator to give a dichloromethane extract ($TaCH_2Cl_2$; 3 mg)

2.4. Phytochemical study

Phytochemical analysis of *T.alnifolia* leaves extracts was carried out by qualitative methods and flavonoids, tannins, leucoanthocyans, carotenoids and saponosides, contents of the *T.alnifolia* leaves were investigated..

Isolation and identification: The methanolic residue of *T.alnifolia* leaves (3 g) was applied to a Sephadex LH-20 column and eluted with ethanol. The chromatographically identical fractions, as assessed by thin layer chromatography (TLC) monitoring, were combined. Fraction 2, which was rich in flavonoids, was subjected to repeated column chromatography and TLC (ethyl acetate/acid formic/H20 (30:1:20)) resulted in 5 mg of compound which was submitted for identification by NMR. The NMR spectra while recorded in MeOD using a Varian 400 MHZ spectrometer at University of Antwerp.

2.5. Biological testing

The antimicrobial activity (antibacterial, antifungal, antitrypanosomal and antiplasmodial) was evaluated according to previous efforts (Baldé et al., 2010; Baldé et al., 2020; Cos et al., 2006). Briefly, an integrated screening concept for anti-infective activity was applied in which antibacterial, antifungal, and antiparasitic 'whole organism' assays, as well as cytotoxicity on MRC-5 cells (human lung fibroblasts) are performed in parallel. Standardization across the different bioassays maximizes efficiency, minimizes cost, and allows easy and reproducible data acquisition. It included the use of standard (20 mM or 20 mg/ml) stock solutions in 100 % DMSO; fixed concentrations in all screens (using 2- or 4-fold serial dilutions); standard layout of 96-well microplates to facilitate plate production and to minimize human errors during the bioassay, always including negative, positive and reference controls; spectrophotometric reading of endpoints, whenever possible; and standard spreadsheet templates, thereby allowing rapid result processing and reporting.

2.5.1. Antibacterial and antifungal screening

The following strains of bacteria and fungi were used: *Escherichia coli* ATCC-8739, *Staphylococcus aureus* ATCC-6538 *Mycobacterium chelonae* ATCC 35752 and *Candida albicans* ATCC-10231. Estimation of the minimal inhibitory concentration (MIC) was carried out by the broth dilution method. 100 μL of a bacterial or yeast suspension was aliquoted into 96-well plates and subsequently 100 μL of a twofold plant extract dilution was added to each well at desired concentrations. A control for normal microbial growth consisting of media without extract was added. Culture broths used were tryptic soy broth (TSB) for bacteria and Sabouraud (SDA) for fungi. The wells were inoculated with a microbial suspension of 10^5 CFU/mL and then incubated for 24 h. The inhibition of bacterial or yeast growth was evaluated by comparing wells with extracts to wells with normal microbial growth in the control wells without plant extracts. The minimal inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited macroscopic growth of bacteria or yeast. Samples with a MIC value of < 64 μg/mL were considered active. Ampicilin (Fluka), rifampicin (Fluka) and flucytosine (Sigma) were used as positive controls. These reference compounds are routinely used in the screening platform, and their activities were in the range that is usually observed.

2.5.2. Antitrypanosomal activity

2.5.2.1. Trypanosoma brucei. Briefly, Trypomastigotes of *T.brucei* Squib-427 strain (suramin-sensitive) were cultured at 37 °C and 5 % CO_2 in Hirumi-9 medium, supplemented with 10 % fetal calf serum (FCS). Assays were performed by adding 1.5×10^4 Trypomastigotes/well. After 72 h incubation, parasite growth was assessed fluorometrically by adding resazurin for 24 h at 37 °C. Fluorescence was measured using a Genios Tecan fluorimeter (excitation 530 nm, emission 590 nm). Suramin was included as a reference drug.

2.5.2.2. Trypanosoma cruzi. Tulahuen CL2 strain (nifurtimox-sensitive) was maintained on MRC-5 cells in minimal essential medium (MEM) supplemented with 20 mM l-Glutamine, 16.5 mM sodium hydrogen carbonate and FCS (5 %) at 37 °C and 5 % CO_2. To determine *in vitro* Antitrypanosomal activity, 4×10^3 MRC-5 cells and 4×10^4 parasites were added to each well of test plate with compound. After incubation at 37 °C for 7 days, parasite growth was assessed by adding of beta-galactosidase substrate, chlorophenol red beta-d-galactopyranoside for 4 h at 37 °C. The color reaction was read at 540 nm and absorbance values were expressed as a percentage of the blank controls. Nifurtimox was included as a reference drug.

2.5.3. Antileishmanial activity

Leishmania infantotum amastigotes (MHOM/ET67) were collected from an infected donor hamster and used to infect primary peritoneal mouse macrophages as described (Corral et al., 2013). To determine *in vitro* Antileishmanial activity, 3×10^4 macrophages were seeded in each well of a 96-well plate. After 48 h outgrowth, 5×10^4 amastigotes/well were added and incubated for 2 h at 37 °C. Pre- diluted compounds were subsequently added and the plates were further incubated for 120 h at 37 °C and 5 % CO_2. Parasite burdens were determined microscopically after Giemsa staining and expressed as a percentage of the blank controls without compound. Pentostam was included as reference drugs.

2.5.4. Antiplasmodial activity

Antiplasmodial activity evaluations were carried out as previously described (Baldé et al., 2010). The chloroquine-susceptible *P. falciparum* GHA- strain was used. Parasites were cultured in human erythrocytes A+ at 37 °C under a low oxygen atmosphere (3 % O_2, 4 % CO_2, and 93 % N_2) in a modular incubation chamber.The culture medium was RPMI-1640, supplemented with 10 % human serum. 200 μL of infected human red blood cells suspension (1 % parasitemia, 2 % hematocrit) were added to each well of the plates with test compounds and incubated for 72 h. After incubation, test plates were frozen at −20 °C. Parasite multiplication was measured by the Malstat method. 100 μL of Malstat reagent were transferred in a new plate and mixed with 20 μL of the haemolysed parasite suspension for 15 min at room temperature. After addition of 20 μL nitroblue tetrazolium (NBT)/ phenazine ethosulfate (PES) solution and 2 h incubation in the dark, the absorbance was spectrophotometrically read at 655 nm using a Biorad 3550-UV microplate reader. Percentage growth inhibition was calculated compared to the negative blanks. Artesunate and chloroquine were included as reference drugs.

2.5.5. Anti-HIV assay

Anti-HIV screening against HIV-1 (strain IIIb) and HIV-2 (strain ROD) was carried out as reported before (Maregesi et al., 2010). Testing of anti-HIV activity used is based on evaluation of the inhibitory effect of a test agent against the cytopathic effects of the HIV-virus in a T-cell model using MT-4 cells. Azidothymidin was used as a positive control.

2.5.6. Anti-SARS-CoV-2 assay

Vero-E6 cells were obtained from the American Tissue Culture Collection and cultured in D10+ medium consisting of Dulbecco's Modified Eagle Medium, 10 % fetal bovine serum (Gemini Bio Products, West Sacramento, USA), 100 U of penicillin/mL, and 100 μg of streptomycin/mL (Sigma Aldrich, St. Louis, USA) at 37 °C and 5 % CO_2. The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281. To generate virus stocks, 3×10^6 Vero-E6 were incubated in 15 mL of D10+ for 24 h, replaced with fresh media, and incubated with virus at a multiplicity of infection of 0.001. Cells were incubated for 5–7 days until clear cytopathic effect was observed. Media was harvested and stored at −80 °C. To determine virus titers, cells were plated in 96-well format at 2×10^4 cells/mL, incubated for 24 h, and then washed and incubated in fresh media containing 5-fold serial dilutions of thawed virus aliquot for 4 days. Wells were then scored visually for the presence of cytopathic effect, and 50 % tissue culture infectious dose $TCID_{50}$ was calculated using the Reed-Muench method.

Antiviral screening was performed as described in Tietjen et al., 2021. Briefly, Vero-E6 cells were plated in D10+ medium at 2×10^4 cells/mL in 96-well format, and extracts were added to cells at stated concentrations in triplicate. After 2 h, cells were infected with 150x $TCID_{50}$ of SARS-CoV-2 (USA-WA1/2020 variant). After 4 days incubation, cells were treated with resazurin to a final concentration of 20 μg/mL and incubated for an additional 4 h. Cells were fixed with 4 % paraformaldehyde for 30 min to inactivate virus, and cell viability was measured using a ClarioStar plate reader (BMG Labtech) (Tietjen et al., 2021). Remdesivir was included as a reference control. To determine effects of extracts on cell viability in the absence of infection, uninfected cells were cultured in parallel as described.

Table 1
Sociodemographic data of healers.

Characteristics Age groups	Number of healers	Percentage (%)
30–40	9	23.1
41–50	13	33.3
51–60	11	28.2
> 60	6	15.4
Total	39	100.0
Sex		
Male	16	41.0
Female	23	59.0
Total	39	100.0
Mode of acquisition of knowledge		
Mode of acquisition		**Percentage** (%)
Family legacy	33	84.6
Learning	4	10.3
Former patients	1	2.6
Dreams	1	2.6
Total	39	100.0

Table 2
Methods of preparation and administration of recipes.

Recipe preparation mode		
Decoction	13	65.0
Powder	7	35.0
Total	20	100.0
Recipes administration method		
Oral	3	14.3
Bath followed by oral intake	9	42.9
Local application	7	33.3
Oral followed by local application	2	9.5
Total	21	100.0

3. Results

3.1. Ethnomedical survey

The results of the ethnomedical surveys are shown in Tables 1–3.

From the ethnomedical survey, *Tetracera alnifolia* was also found to have numerous uses in Guinean traditional medicine (Table 3).

3.2. Phytochemical study

The phytochemical screening indicated the presence of flavonoids, tannins, leucoanthocyans, carotenoids and saponosides in the leaves extracts. From the methanolic extract, one isolated compound was identified as Kaempferol on the basis of its NMR data which were in agreement with the literature data (Oriakhi et al., 2022).

Summary of ^1H and ^{13}C NMR data for compound identified (400 MHZ): 1H: δ= 6.44a (d, J = 2.4HZ); 6.82(d, J = 8.8), 8.03(d, J = 2HZ), 6.91(d, J = 2.8HZ), 6.91a' (d, J = 2.8HZ), 8.03(d, J = 2HZ). 13C: δ= 129.0 (C-2), 123.2(C-3), 183.9(C-4), 159.4(C-5), 100.6(C-6), 166.6(C-7), 80.5 (C-8), 130.0(C-9), 101.3(C-10), 116.3(C-1′), 117.0(C-2′), 105.1(C-3′), 151.7(C-4′), 105.1(C-5′), 117.0(C-6′).

Table 3
Descriptions of diseases according to healers and ethnomedicals uses of *T. alnifolia*.

Vernacular name	Language	Symptoms treated	Approximate correlation	Number of quoted healers
Makourou	Maninka / Sosso	Warming of the belly and pain, pimples on the body, abdominal pain, stomach ache	Pyoderma	4
Gnoni	Maninka	Warming of the body, eyes and tongue are red and very painful	Varicella	1
Wagna	Maninka	Buttons, itching	Scabies	5
Cassi	Sosso	Itching, small pimples	Scabies	2
Makourou yègbè	Sosso	Fever and the body part is red	Pyoderma	2
Cassi khounkhouri	Sosso	Small pimples and itching	Scabies	1
Soukhou kouyé	Sosso	White fluid loss, pain on urination, itching, unpleasant odour in women.	Urogenital infections	18
Fassa	Maninka	Significant weight loss with the appearance of nerves on the skin in children.	Marasmus in children	22
Konobori	Maninka	Stools passed several times with fluid loss, sometimes with or without fever.	Diarrhea	3
Temou Banki	Sosso	Sex doesn't get up	Erectile dysfunction	5
Mangué fakhè	Sosso	Stomach ache, pain, constipation, anal itching, sometimes bleeding.	Hemorrhoids	3
Danawali	Sosso	Yellow tegument, fever, headache, chills, fatigue, vomiting	Malaria	2
Faté fountou	Sosso	Soft tissue swelling, sometimes a little painful	Heart failure	2
Takoé	Sosso	Whitish deposit on baby's tongue, pain, greenish stools.	Oral candidiasis	2
Koundimi gbèlè	Maninka	Severe head pain, difficulty opening eyes.	Sinusitis	1
Sorom dimi	Maninka	Pain in the pelvic region	Kidneydisease	1
Gnama	Maninka	Lumbago with bleeding, nausea, headache, cramp in women.	Dysmenorrhea	2
Fassa dimi	Sosso	Pain, numbness, burning sensation in hands or feet	Neuropathy	1

3.3. Biological activities

3.3.1. Antibacterial and antifungal activities

Three extracts (Chloroformic, methanolic and aqueous) were tested for antimicrobial activities. The antimicrobial effects of the extracts are summarized in Table 4. Aqueous and chloroformic extracts were not active against bacterial and fungal strains tested, while the methanolic extract was active against the *Staphylococcus aureus* strain (MIC = 29.9 μg/mL).

3.3.2. Anti-plasmodial, antileishmananial and anti trypanosomial activities

Chloroformic, methanolic and aqueous extracts were next tested for antiprotozoal activity as summarized in Table 5. None of tested extracts were active against *Plasmodium falciparum* strain, while chloroformic extract was active against both trypanosma and leishmania strains.

Table 4
in vitro antibacterial and antifungal activities of extracts *T.alnifolia* (MIC in μg/mL).

Samples	Sa	Ec	Ca	Mc
$TaCHCl_3$	> 64 **μg/ml**	> 64 **μg/ml**	> 64 **μg/ml**	> 64 **μg/ml**
Ta CH_3OH	29.9 **μg/ml**	> 64 **μg/ml**	> 64 **μg/ml**	> 64 **μg/ml**
TaH_2O	> 64 **μg/ml**	> 64 **μg/ml**	> 64 **μg/ml**	> 64 **μg/ml**

Sa: Staphylococcus aureus; Ec: E. coli; Ca : Candida albicans ; Mc : Mycobactérium chelonae.

Table 5
anti-trypanosomiasis, anti-leishmanial and anti-plasmodial activities *in vitro* extracts of *T .alnifolia*.

Samples	*T.cruz*	*T.brucei*	L.*inf*	*Pf-GHA*
$TaCHCl_3$	40.0 μg/ml	20.2 μg/ml	32.5 μg/ml	> 64 μg/ml
Ta CH_3OH	>64 μg/ml	28.2 μg/ml	8.1 μg/ml	> 64 μg/ml
TaH_2O	> 64 μg/ml	> 64 μg/ml	> 64 μg/ml	> 64 μg/ml
Chloroquin				0.05 μM
Benznidazol	2.0 μM			
Suramin		0.04 μM		
Miltefosine			6.1 μM	

T.cruz: Trypanosoma cruzi; L.*inf: Leishmania infantotum; T.brucei: Trypanosoma brucei; Pf- Gha: Plasmodium falciparum*-Ghana.

3.3.3. Anti HIV activity

With regard to HIV, the chloroformic, methanolic and aqueous extracts showed MICs of 57.7, 62.7, and 52.6 μg/mL, respectively against HIV-1 IIIB, with less overall toxicity in MT-4 cells, in all cases with an index of selectivity > 2. None of extracts tested was active against HIV-2.

3.3.4. Anti SARS-COV-2 activity

Chloroformic extract, methanolic and dichloromethane extracts were also tested for ability to inhibit live SARS-CoV-2 replication (parental Wuhan variant) in Vero-E6 cells (Fig. 1). In this assay, chloroformic extract inhibited up to 31.4 ± 15.5 % (mean ± *s*.e.m.) of virus replication relative to untreated, infected cells at 25 μg/mL, while extensive cell death was observed at 50 μg/mL. For dichloromethanic extract, up to 53.4 ± 5.4 % of virus replication was inhibited at 50 μg/mL. In uninfected cells assessed in parallel, no toxicity was observed (*i.e.*, > 90 % viability relative to untreated cells) at any concentration up to 50 μg/mL (Fig. 1), indicating good *in vivo* tolerability of these extracts. The additional toxicity observed in infected cells treated with 50 μg/mL of chloroformic extract, relative to uninfected cells, is therefore likely to reflect an additive effect of both virus infection and high concentration of chloroformic extract.

4. Discussion

Herbal medicine is a practice widely used by the population of Guinea for the management of several pathologies. This ethnomedical survey was conducted in some markets of the city of Conakry on the traditional use of *Tetracera alnifolia*. In total, we identified 39 healers, including 23 women (59.0 %; Table 1) who participated in our survey. Indeed, this predominance of women is justified by the fact that women tend to be more interested in traditional medicine in this area. This result is somewhat different from that of Magassouba et al. (2007), who found a male predominance of healers (56.6 %).

The age of healers was between 30 and 80 years old, and the most represented group was 41 to 50 years old with a frequency of 33.3 % (13/39), followed by 51 to 60 years old with a frequency of 28.2 % (11/39) (Table 1). This is in agreement with the previous results described in Guinea (Diallo et al., 2012; Magassouba et al., 2007). These results show that young people are less likely to be involved in the trades of traditional healers and indicates a need to promote traditional medicine among youth to prevent a gradual disappearance

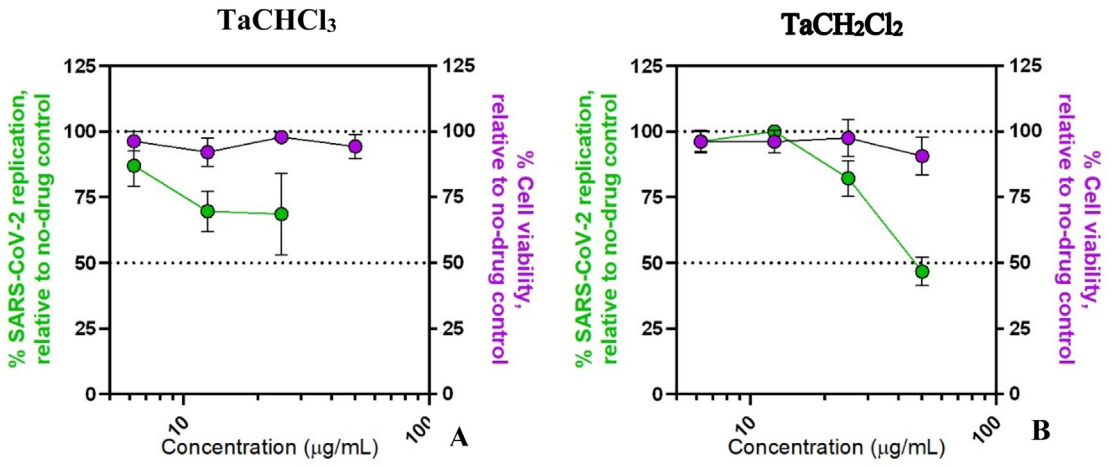

Fig. 1. Dose response curves of chloromethanic extract A (left) and dichloromethanic extract B (right) on viral replication in SARS-CoV-2 USA-WA1/2020 variant-infected Vero-E6 cells (green) and cell viability in uninfected Vero-E6 cells (purple). Data are presented as percent SARS-CoV-2 replication and percent cell viability relative to uninfected or untreated cells, respectively, with 0 % denoting viability of infected cells without treatment (for infection experiments) or media-only control (for cell viability experiments). Results show the mean ± s.e.m. of three independent experiments.

of this protected traditional knowledge. We also noted in our survey that 71.8 % (28/39) of healers were not enumerated by the Ministry of Health compared to 28.2 % (11/39) that were previously identified. This census deficit makes it difficult to organize and control the activities of healers, as well as to preserve and standardize their knowledge, and suggests that additional advocacy for healer organizations in Guinea is needed. The transmission of this traditional art is mainly inherited (84.7 %, or 33/39) (Table 1). This result is similar to that reported by Traoré (Traore et al., 2013), who in an ethnobotanical survey on malaria in Guinea, reported a predominance of the acquisition of traditional art by inheritance of 50 %, while acquisition by learning was much less common, *i.e.* 10.3 % (4/39)

Tetracera alnifolia have applications Guinean traditional medicine for numerous ailments and including infections, which was further supported by descriptions of diseases treated with *T. alnifolia* according to our survey of healers in Conakry (Table 3). During our investigation, we identified six (6) pathologies mentioned by the healers of which the most cited were "*Wagna*/ Scabies " (5 quotes), "*Makourou*/ Pyoderma " (4 quotes), "*Cassi*/ Scabies " (2 quotes) and "*Makourou yègbè*/ Pyoderma "(2 quotations) (Table 2). According to the descriptions of these pathologies by the healers, these affections could be of allergic origin (*Cassi khoun khouri, Wagna*). The most cited method of preparation was decoction (13 citations) followed by powder (7 citations) (Table 1). This result is similar to that of Diatta et al**.;** in their study carried out on the medicinal plants used against dermatoses in the Baïnounk pharmacopoeia of Djibonker, Senegal where the decocted ones were the most cited (Diatta et al., 2013). A decoction would be expected to collect the most active ingredients and mitigate or cancel the toxic effect of certain recipes (Zhang et al., 2018). Mainly, these forms of presentations, although easy to prepare, have certain disadvantages, including poor conservation, which results in the appearance of pathogenic molds and microorganisms in the recipes over time. We noticed in our investigation that *T. alnifolia* is also used in the treatment of other pathologies apart from dermatological conditions. These include "*Fassa*" (marasmus or malnutrition), "*Tèmou bankhi*" (sexual weakness in men) and "*Soukhou kouyé*" (white losses in women) (Table 3).

Analysis of the phytochemical screening of the aqueous extracts of *T alnifolia* revealed several classes of secondary metabolites including cardiotonic heterosids, flavonoids, and saponosides as well as steroids, alkaloids, terpenoides, and tannins (Ogunlakin and Sonibare, 2022). Preliminary analysis from NMR data support the presence of Kampferol in *Tetracera alnifolia*. Kampferol-7-O-glucoside, a flavonoid glycoside, has been reported to exhibit antimicrobial and antioxidant activities (Ogbole et al., 2017). Kaempferol and its associated compounds as knowed to exhibit antibacterial, antifungal, and antiprotozoal activities. (Periferakis et al., 2022)

Three extracts of *T.alnifolia* were tested *in vitro* against microorganisms. Among the tested extracts, only the methanolic one presented a modest activity against *S. aureus*. Activity of methanolic extracts maybe du to Kaempferol. So kaempferol is known to inhibit the activity of *S. aureus* PriA helicase (SaPriA) and the activity of bacterial efflux pumps, thereby blocking the growth and survival of antibiotic-resistant *S. aureus* and increasing antimicrobial effectiveness (Ming et al., 2017).

None of these extracts was active against *Plasmodium falciparum*-Ghana. Previous investigations on biologically active plants have shown that crude extracts are usually more active than individual constituents of the plant (Rasoanaivo et al., 2011). However, both chloroformic and methanolic extracts of *T.alnifolia* moderately inhibited tested protozoa (*Trypanosoma* and *Leishmania*). The antiprotozoal that we observe here is in accordance with results reported by Traore et al. (2014). The lack of novel drugs for animal and human trypanosomiasis remains chronic and acute. Drugs resistance may perhaps best be countered by biologically active crude extracts from plants rather than the use of combinations of single chemical drugs known to be effective despite the risks of dual resistance (Cheuka et al., 2016) Available drugs for human trypanosomiasis now seem to be under production but have significant restrictions and are administrable with reasonable safety only under hospitalization (Kasozi et al., 2022)

Among the extracts tested in HIV, only the aqueous extract one presented a weak effect against HIV-1 IIIB strain. This weak activity could be du to presence of Kampferol which possesse anti-HIV-1 reverse transcriptase activity (Behbahani et al., 2014)

In contrast, two apolar extracts (Chloroform and dichloromethane) inhibited live SARS-CoV-2 replication at 12.5 to 50 μg/mL, depending on the extract, indicating the potential to identify natural product-based antivirals from this source. The ORF3a gene of SARS−CoV encodes a membrane ion channel (3a channel) which is involved in the viral release process from the host cell. The activity of this channel causes a membrane depolarization and the activation of Ca2+ channels, which allows the virus release by exocytosis. This 3a ion channel is inhibited by the kaempferol (Goris et al., 2021).

5. Conclusion

This study, whose purpose was to contribute to the enrichment of the ethnomedical, biological and phytochemical data of *Tetracera*

alnifolia, made it possible to contact 39 healers who frequent markets in Conakry. The ethnomedical survey revealed that the plant is used in the traditional treatment of several diseases including skin infections, marasmus, white discharge in women and sexual weakness in men. Biotic investigations of the chloroform, methanol and aqueous extracts of the leaves showed good activity on protozoa including *Leishmania infantum* and Trypanosoma *brucei*. The methanolic extract showed moderate activity on *Staphylococcus aureus*, while aqueous and dichloromethane extracts acted on HIV-1 and SARS-CoV-2, respectively. Taken together, these studies indicate that *Tetracera alnifolia* extracts have wide traditional use in Guinea and a variety of anti-infective properties *in vitro*.

Declaration of competing interest

All authors declare that there are no conflicts of interest

CRediT authorship contribution statement

A.K. Camara: Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **E.S. Baldé:** Writing – original draft, Supervision, Investigation, Conceptualization. **M.S.T. Diallo:** Writing – original draft, Methodology, Investigation. **M.K. Camara:** Writing – original draft, Methodology, Investigation. **T.V. Bah:** Methodology, Investigation. **M. Condé:** Methodology, Investigation. **A. Soumah:** Methodology, Investigation. **I. Tietjen:** Writing – original draft, Methodology, Funding acquisition, Conceptualization. **A.M. Baldé:** Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2025.04.014.

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