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# Managing sexually transmitted infections in Benin: An ethnopharmacology study among traditional medicine practitioners and *in vitro* activity evaluation of prioritized medicinal plants against *Chlamydia trachomatis*

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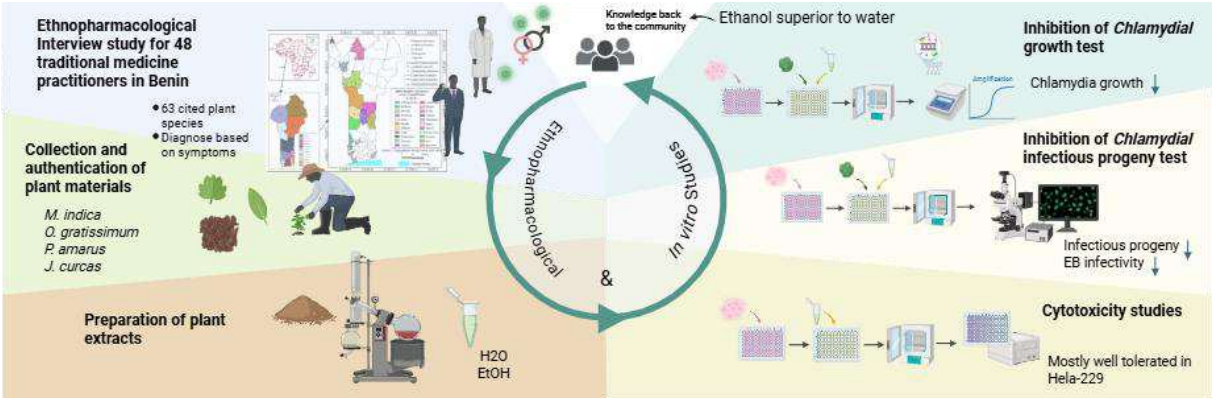
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## Abstract

**Background:** Sexually transmitted infections (STIs) represent a significant health problem in low- and middle-income countries, where access to care is limited and antimicrobial resistance worsens therapeutic outcomes. Medicinal plants and traditional healing practices stand as accessible and culturally acceptable alternatives for STI management, but they remain poorly documented and lack scientific validation. To address this knowledge gap, the present study had two complementary objectives: to document the knowledge, attitudes and practices of traditional medicine practitioners (TMPs) regarding the management of STIs in Benin, and to evaluate *in vitro* the antichlamydial activity of the most frequently cited medicinal plants.

**Methods:** A cross-sectional interview survey was conducted among 48 TMPs originating from 10 departments of Benin, utilizing questionnaires completed with individual discussions with each participant from June to July 2024. Four most frequently cited medicinal plant species were subjected to plant material collection, aqueous and ethanolic extraction and cytotoxicity evaluation in HeLa-229 cells. The antichlamydial activity of the plant extracts was studied by determining *Chlamydia trachomatis* inclusion counts, genomic replication, infectious progeny production and elementary body (EB) infectivity. For data analysis, one-way ANOVA with Dunnett's post-hoc test was used.

**Results:** According to the interview data, the practitioners diagnose STIs through patient-reported symptoms and transmission route as well as their own clinical examination. While the participating TMPs expressed mixed views on collaboration with modern medicine, 71% of them stated guiding patients to formal healthcare in cases of treatment failure or severe symptoms. Regarding treatment practices, complementing medicinal plants with animal or mineral-derived preparations or spiritual rituals were reported by approximately half of the participants. A total of 63 plant species were cited for treating STIs by the survey participants. Ethanolic extracts of all experimentally evaluated plant material exhibited a dose-dependent inhibition of *C. trachomatis* growth, most notably *Jatropha curcas* (100–250 µg/ml, $P = 0.001$-$0.002$, $\eta^2 = 0.799$), alongside significant effects for *Mangifera indica*, *Ocimum gratissimum* and *Phyllanthus amarus* ($P = 0.005$-$0.045$). Genomic replication was similarly reduced ($\eta^2 = 0.775$), as was infectious progeny production ($P = 0.001$–$0.031$, $\eta^2 = 0.851$–$0.942$). *M. indica* extracts additionally suppressed EB infectivity ($\eta^2 = 0.962$), while aqueous extracts showed generally weaker but still significant effects (growth inhibition $P = 0.001$–$0.009$, infectious progeny inhibition 0.006–0.043).

**Conclusions:** Local traditional medicine resources may contribute to STI management in low-resource settings but require support to guide their evidence-based use. Our findings support

the possible efficacy of ethnopharmacologically prioritized plants against *C. trachomatis* and highlight the need for further *in vivo* antimicrobial studies against STI pathogens.

**Keywords:**
Bacterial infection, Traditional medicine, Sexually transmitted infections, *Chlamydia trachomatis*, Ethnopharmacology, Ethnobotany, Benin

## 1. Background

Sexually transmitted infections (STIs) are a major global public health problem, particularly in resource-limited settings where access to modern health care remains a challenge [1]. WHO estimates over 1 million new curable STI cases occurring daily [1]. In addition to chlamydia, gonorrhea, syphilis, and trichomoniasis, widespread viral STIs like human papilloma virus (HPV) and herpes negatively affect sexual and reproductive health, increase HIV transmission risk and cause cancers. In terms of geographic distribution, sub-Saharan Africa bears one of the highest disease burdens with approximately 93 million new STI cases each year [2]. Most STI epidemiological data from sub-Saharan Africa are derived from health records on self-reported cases and show a highly heterogeneous prevalence of STIs. Among sexually active men, reported prevalence rates range from 0.4 % to 13.5 % [3]. In a study focusing on young women in sub-Saharan Africa, the overall prevalence of self-reported STIs was estimated as 6.9 %, with remarkable national differences, with rates ranging from 32.7 % in Mali to 0.3 % in Ghana [4]. Among the bacterial pathogens responsible for STIs, *Chlamydia trachomatis* stands out as the most prevalent. While its overall global prevalence is estimated as 2.9 % (95 % CI: 2.4-3.5 %) [5], in sub-Saharan Africa *C. trachomatis* has been reported with a prevalence of 6.9 % among females and 4.2 % among males, showing 34.5 % increase from 2010 to 2020 [6,7]. Furthermore, a prevalence of 6,1 % among pregnant women has been reported in West and Central Africa, involving a high proportion of asymptomatic cases [8].

Besides its acute symptoms, *C. trachomatis* infection can induce tissue injury and lead to long-term complications in reproductive organs. These include chronic pain, pelvic inflammatory disease, ectopic pregnancies and infertility in women. In neonates, conjunctivitis and pneumonia may occur through vertical transmission. Urethritis, epididymitis, prostatitis, proctitis and reactive arthritis are the most commonly reported consequences of the infection in men [9,10]. Furthermore, *C. trachomatis* infection has been associated with increased risk of HIV transmission by compromising the epithelial barrier and recruiting HIV target cells to the infection site [11-13]. Frequent co-infections of *C. trachomatis* with other viruses, such as HPV

and HSV, have also been documented, and their synergistic interactions are a subject of active investigation [12].

*C. trachomatis* is an obligate intracellular bacterium that primarily infects epithelial cells but can also invade genital tract phagocytes [14]. Its biphasic life cycle alternates between the infectious Elementary Body (EB) and the replicative Reticulate Body (RB) forms. Due to the strictly intracellular replication, host cell membranes cause additional penetration barriers on top of the two cell membranes of the gram-negative bacterium itself [15].

Current treatment guidelines recommend a single dose of azithromycin or a 7-day doxycycline regimen for treating chlamydial infections [15]. While these treatments effectively resolve most acute infections, some studies report treatment failures and limited efficacy in chronic or recurrent cases [15-17]. In addition to the treatment failures arising from compromised clinical practices, poor adherence to medication and lack of treatment for partners, emerging antimicrobial resistance (AMR) is evident as genetic mutations have been detected in *C. trachomatis* strains with reduced susceptibility [18-20].

Regardless of the existence of effective medication to treat chlamydia and other STIs, many African communities continue to rely on traditional medicine (TM) to manage STIs [21]. A vast proportion of African population relies on TM for their primary health care needs [22]. Particularly in rural areas, TM is often more geographically accessible than modern medicine. Cultural factors, including traditional beliefs and social norms, also play a central role [23]. The stigma associated with STIs arising from societal attitudes, misconceptions and internalized self-blame, can refrain individuals from seeking care in modern health facilities [24]. Hence, traditional medicine practitioners (TMPs) are often the first point of contact for people seeking treatment for their STIs and other reproductive health problems.

In the west African republic of Benin, with 10,999 nationally registered TMPs compared to only 489 doctors, the superior accessibility of TM over modern healthcare is evident.

To date, very few studies have documented the management or treatment of STIs within TM practices in West Africa. A few ethnopharmacological surveys involving STIs have been conducted in Nigeria but they focus solely on plants used for STI treatment [25-27], and do not shed light on competence level or professional practices of TMPs.

Despite their key role in managing STIs within the general population, knowledge or practices held by TMPs in STI management have not been documented by scientific research. To address this knowledge gap, the current study had two objectives: 1) to document the TM practices and medicinal plants used by Beninese TMPs to manage STIs and 2) to experimentally evaluate the *in vitro* activity of the most frequently cited plants against *Chlamydia trachomatis*. By exploring

the intersection of local knowledge, biodiversity resources and public health needs, this study seeks to contribute to the integration of TM into local health systems in an evidence-based manner.

## 2. Methods

### 2.1 Study area

The interview study took place in the Republic of Benin from June to July 2024. Benin is a West African country with 13.3 million inhabitants spread over twelve departments and seventy-seven communes. The country is located between latitudes 6°30' N and 12°30' N, characterized by a south-north precipitation gradient and a corresponding diversity of vegetation zones. Benin spans three main climatic zones: the humid Guinean zone in the south (annual rainfall > 1200 mm), the transitional Sudano-Guinean zone in the center (900–1100 mm), and the drier Sudanian zone in the north (600–900 mm). This survey covered 10 departments of Benin, spanning all three climatic zones: Atacora, Donga (northern Sudanian zone); Collines, Zou, Plateau (central Sudano-Guinean zone); Couffo, Mono, Atlantique, Littoral, Ouémé (southern Guinean zone).

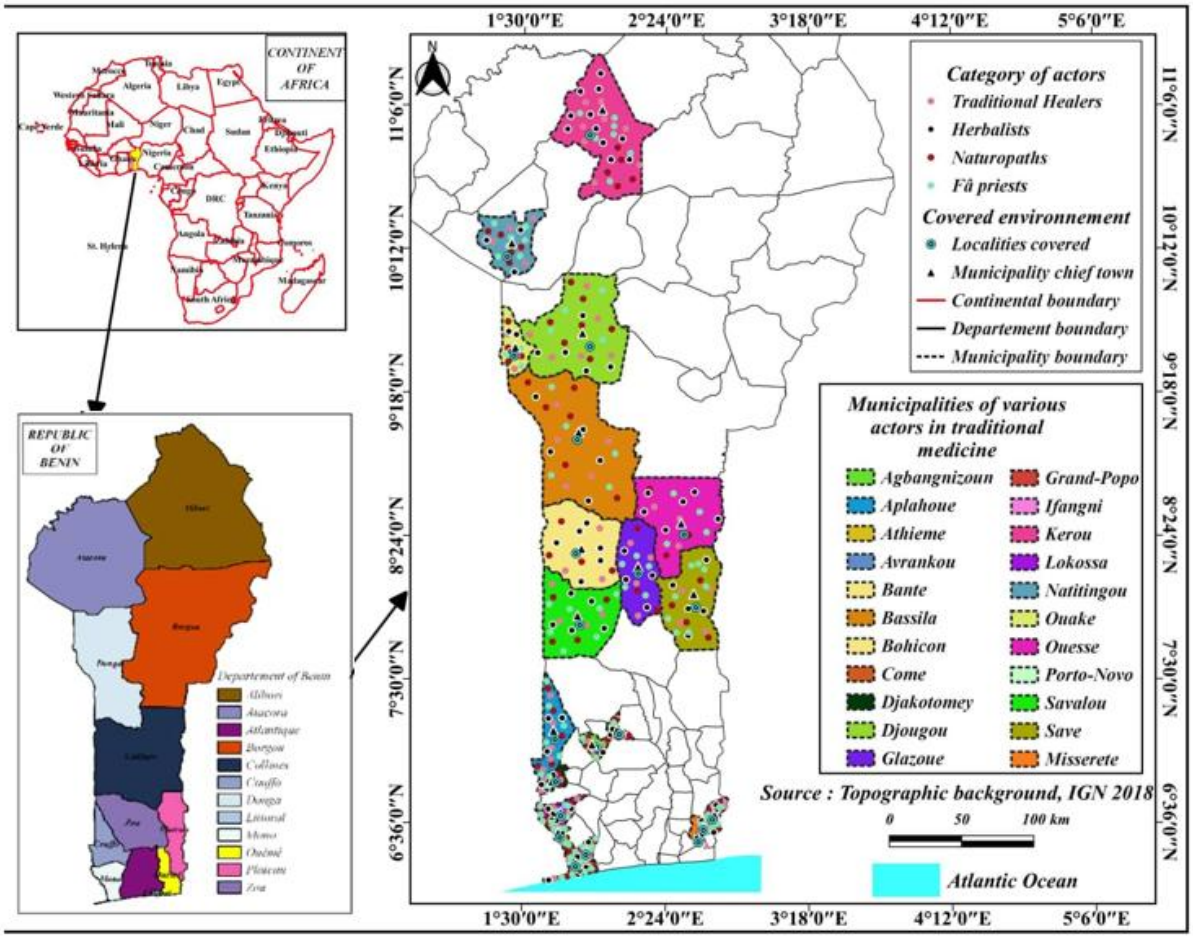


**Figure 1.** Map of Benin showing the Benin departments and communes covered by the survey. Map created with QGIS 3.18 software.

### 2.2. Interview study

The study participants consisted of 48 TMPs invited by the National Pharmacopoeia and Traditional Medicine Program. The inclusion criteria required a legally recognized TMP status in Benin, proven experience in the practice of traditional medicine and the treatment of STIs, and a clear willingness to participate in the study.

The Beninese Ministry of Health, through its National Program of Pharmacopoeia and Traditional Medicine (PN-PTM), acts as the governmental body overseeing the sector and integrity of all activities targeting traditional medicine practitioners. PN-PTM has established interaction with the national associations of the practitioners and before giving its permission for the conduct of any study, consults the representatives of the TMP associations to hear their views and to identify survey participants with relevant professional skills. After obtaining ethics committee approval, we followed this procedure and obtained a list of 70 prospective survey participants. Aiming for best possible diversity in terms of participant experience level and geographic distribution within the country, we contacted 60 out of these 70 persons. Due to a lack of availability or willingness to participate (e.g., in the case of 12 TMPs), the final number of study participants was 48. The participating TMPs come from 27 communes, shown in Figure 1.

Regarding data collection, a semi-structured interview was utilized through individual discussion in June to August 2024 [28,29]. Prior to data collection, the questionnaire was validated through discussion and feedback with three TMPs not participating in the actual study, focusing on making the questions understandable for traditional medicine context. The survey was conducted using the KoboCollect application, using the questionnaire provided in Supplementary File 1. The collected data covered socio-demographic characteristics, knowledge on STIs, diagnostic and treatment practices, medicinal plant species and organs used, preparation methods, routes of administration, dosages, and efficacy indicators.

Interviews were mainly conducted in local languages (Fon, Goun, Yoruba, and Adja), depending on the region, with some conducted in French in the Atlantic and Littoral departments. Local languages were preferred in the discussions to avoid misunderstandings and to facilitate communication with participants. Each interview was conducted by two

researchers, at least one of them being a native speaker of the language used for the conversation. During the interview, answers were recorded in writing in French.

### 2.3 Plant material

After the interview survey and analysis of the results, organs of the medicinal plants selected for experimental work were collected in the municipality of Abomey-Calavi, more specifically in the district of Pahou. The bark and leaves of *Mangifera indica*, leaves of *Phyllanthus amarus*, *Ocimum gratissimum* and *Jatropha curcas* used in this study were collected directly from nature in October 2024. The exact collection site was geo-referenced as follows: Latitude 7.2900935; Longitude 2.1183021; Altitude 22.6 m; with a location precision of 2.2 m. No specific permits were required for collection, as the area is not under a protected status and none of the collected species are classified as vulnerable or endangered. The plant material was authenticated at the National Herbarium of Benin under the numbers as follows: *Mangifera indica*: YH385/HNB, *Phyllanthus amarus*: YH359/HNB, *Ocimum gratissimum*: YH325/HNB and *Jatropha curcas*: YH379/HNB. Authentication was done by Professor Yedomonhan Hounnankpon, curator of the Benin National Herbarium. The botanical nomenclature of Plants of the World Online website (www.worldfloraonline.org) was used to confirm the complete names of all medicinal plants listed in this study.

To eliminate potential microbial contaminants and surface impurities, the plant material was washed with a 1/100 bleach solution, extensively rinsed with clean water and allowed to dry. After drying at room temperature, each plant material was ground, and the powders were subjected to microbiological quality control based on European Pharmacopoeia 2.6.12 (Ph. Eur. 2026a) and the results were analyzed based on quality criteria 5.1.8 (Ph. Eur. 2026b).

### 2.4 Plant material extraction

Ethanolic and aqueous extracts were prepared from the five selected plant organs. A total of 25 g of dried and crushed plant material was mixed with 250 ml of solvent [ethanol at 99.5% for ethanolic extract (Anora group Oy, Finland) and Milli-Q water for aqueous extract] and sonicated for 1 h. Suspensions were filtered through Whatman No. 1 paper and the supernatants then centrifuged at 400 $\times$ *g* for 15 minutes. Solvent was evaporated with rotavapor and extract was freeze dried overnight with Lyovapor L-200 Pro (Buchi, Flawil, Switzerland). The extracts were stored at -20°C until further use. For experiments, stock solutions were prepared in filtered dimethyl sulfoxide (DMSO, Thermo Scientific, Vantaa, Finland) at concentration of 50 mg/ml.

204 **2.5 Cell culture**

205 Hela-229 (ATCC CC-2.1) cells were maintained in DMEM medium (Cytiva, Utah, USA)
206 supplemented with 10% FBS (BioWhittaker, Lonza, Walkersville, USA), 2 mmol/L L-
207 glutamine (BioWhittaker, Lonza, Walkersville, USA), 1 mmol/L sodium pyruvate (Sigma
208 RNBM9356, Buchs SG, Switzerland) and 20µg/ml gentamycin (Sigma, Massachusetts, USA).
209 For experiments, cells were seeded into well plates at indicated densities and allowed to adhere
210 overnight before each experiment.

211 **2.6 Cytotoxicity assay**

212 For cytotoxicity assays, Hela-229 cells were seeded into 96-well plates at density of $2 \times 10^4$
213 cells/well and allowed to adhere overnight. Culture medium was replaced with fresh aliquots
214 supplemented with the plant extracts at final concentrations ranging from 5 to 250 µg/ml, the
215 concentrations being selected based on expected physiologically achievable levels. After 48 h
216 incubation, cells were washed with phosphate-buffered saline (PBS, in house made) and 20
217 µmol/L resazurin (Sigma Aldrich, Shanghai, China) solution was added. After 2 h incubation,
218 fluorescence was recorded at 570/590 nm with Varioskan Lux plate reader (Thermo Fisher
219 Scientific, Vantaa, Finland).

220 **2.7 *Chlamydia trachomatis* infection**

221 For infection experiments, HeLa-229 cells were seeded into 24 well plates at density of $4 \times 10^5$
222 cells/well and allowed to adhere overnight. The resulting confluent monolayers were inoculated
223 with *C. trachomatis serovar* K (ATCC VR-887), propagated in Hela-229 cells as previously
224 described by Cebollada et al. [30] at multiplicity of infection (MOI) 0.5 in cell culture medium
225 supplemented with 1 µg/ml cycloheximide (CHX, Sigma, Massachusetts, USA). For each
226 extract, two different concentrations were tested. The concentrations were determined on the
227 basis of the results of the cell cytotoxicity assay. For this study, the concentrations tested range
228 from 50 µg/ml to 250 µg/ml. The plates were centrifuged at 550 × *g* for 1 h and further incubated
229 1 h at 37 °C. Then, the inoculation medium was removed, and the studied plant extracts were
230 added diluted in fresh CHX supplemented medium. In all chlamydial infections, azithromycin
231 (Cayman Chemicals, Ann Arbor, Michigan, USA) at concentration of 40 nmol/L was used as
232 positive control. The cultures were incubated for 46 hours before collecting samples.
233 For immunofluorescence detection of chlamydial inclusion counts, HeLa-229 cells were plated
234 into the 24 well plate with cover slides and at 48 h post infection, cells were fixed with methanol
235 (Sigma, Wisconsin, USA) and chlamydial titers were determined by staining the inclusions with
236 a genus specific antibody. The cover slides were blocked with 0.5% bovine serum albumin

(BSA, Sigma, Missour, USA) solution. The blocked cover slides were incubated with 1:100 dilution of anti-Chlamydia LPS Monoclonal Antibody (B410F, Invitrogen, Illinois, USA) in 0.2% BSA for 1 h, washed three times with PBS and incubated 1 h with 1:2000 dilution of Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody (Alexa Fluor™ 488, Invitrogen, Oregon, USA) in 0.2% BSA. Finally cover slides were washed with milliQ water and inclusions detected using EVOS FL fluorescence microscope (Thermo Fischer Scientific, Life technologies, CA, USA).

### 2.8 Infectious progeny and genome copy number determination

To quantify chlamydial infectious progeny production, Hela-229 cells were infected and treated with the plant extracts as described above. At 48 h post infection, intracellular EB progeny was harvested by scraping the cells from the wells into fresh cell culture medium supplemented with CHX. Infectious progeny was determined by infecting fresh Hela-229 cell monolayer with collected cell lysates. After infection, inoculum was removed, replaced with CHX supplemented cell culture medium and cells were incubated 46 h. Inclusions were determined by staining with chlamydia specific antibody as described above.

To determine *C. trachomatis* genomic replication, cells were harvested 48 h post infection and DNA was purified with GeneJET Genomic DNA Purification Kit (Thermo Fisher, Vilnius, Lithuania). Chlamydia genome copy numbers were determined by qPCR in SyberGreen mastermix (Thermo Fisher Scientific, Vilnius, Lithuania) reaction with OmpA primers (F:5'-GGC GTA TTT GGG CAT CCG AGT AAC G-3' C, R: 5'- TCA AAT CCA GCG GGT ATT AAC CGC CT-3') as described before [31,32].

### 2.9 EB infectivity assay

To evaluate the impact of plant extracts on *C. trachomatis* EB infectivity, EB suspensions were incubated for 1 h in cell culture medium supplemented with ethanolic plant extracts at 4 °C. After incubation, suspensions were centrifugated at 21,000 × *g* for 1 h at 4 °C to separate EBs. The EBs were re-suspended to fresh CHX supplemented medium and used to infect Hela-229 cells as described above. At 24 h post infection, cells were collected for DNA extraction and genome copy numbers were determined with qPCR.

### 2.10 Data analysis

Data processing was carried out using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA). Statistical analysis was carried out using SPSS Statistics 25 software (IBM Corporation, Armonk, NY, USA). Normality of data distribution was assessed prior to statistical testing. Continuous variables with normal distribution are presented as mean ± standard error

of the mean (SEM); ethnopharmacological survey data are presented as absolute and relative frequencies (percentages). Between-group comparisons of chlamydial inclusion counts, genome copy numbers and infectious progeny were performed using one-way ANOVA, with Dunnett's test applied as post-hoc test to compare each extract-treated group against the untreated (vehicle) control. CC50 values were determined by non-linear regression using GraphPad Prism 9.0. For descriptive analysis of the ethnopharmacological data and to prioritize medicinal plant species for experimental evaluation, Relative Frequencies of Citation (RFC) were calculated [33]. Statistical significance was set at $P < 0.05$ (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). At least two individual experiments were carried out for each set of data.

## 3. Results

### 3.1. Socio-demographic data of the participating traditional medicine practitioners

Among the TMPs registered by the Beninese Ministry of Health, different professional subgroups can be distinguished based on the nature of their practice. The majority of the participants in this study (62.5%) self-identified themselves as traditional healers, while fewer phytotherapists (31.2%) and herbalists (6.2%) also took part. Traditional healers are practitioners who use a so-called holistic approach, combining spiritual elements and medicinal plants to treat illnesses. Their practice may include the use of medicinal plants, animal products, minerals, as well as spiritual or cultural rituals. Phytotherapists are health practitioners who specialize specifically in the use of plants and plant-based preparations for therapeutic purposes. Herbalists are practitioners who sell plant materials to people to promote health and treat illnesses, but their practice is usually less formalized than that of phytotherapists or traditional healers. Most survey participants were men (85.5%), aged 31– 40 years (31.2%) and 41– 50 years (27.1%). While all participants reported having some level of formal education, only 29.2% reached higher education levels. Most were married (91.7%) and identified as animists (39.6%), Christians (29.2%) and/or Muslims (27.1%). Fon ethnic group members dominated (39.6%), followed by Adja (22.9%) and Yoruba (12.5%). In terms of experience, 25.0% had been practicing their profession for over 20 years, with 81.3% acquiring knowledge through family transmission and 14.6% through training by a TMP not being their close family member. Comprehensive sociodemographic data of the 48 participants are presented in supplementary table 2.

### 3.2. Knowledge and diagnostic practices on sexually transmitted infections among traditional medicine practitioners

The TMPs in the study demonstrated varying levels of knowledge about STIs. All participants stated that they are aware of STIs, with 83.3% defining them as infections transmitted through unprotected sexual contact (vaginal, anal, or oral) caused by bacteria, viruses, fungi, or parasites (Table 1). When describing the typical features of STIs, respondents cited symptoms including pain during urination (mentioned by 33.3%), vaginal discharge (29.2%), pain during sexual intercourse (18.8%), and genital itching (2.1%). However, 16.7% of the participating practitioners were unaware of STI symptoms and could not mention any descriptive symptoms for them.

**Table 1:** Participants' knowledge of sexually transmitted infections (STI), reported symptoms and perceptive difference between STIs

| Topic inquired | Answers by TMPs | Percentage (%) |
|---|---|---|
| TMP's familiarity with STIs | Yes | 100 |
| | No | 0.0 |
| What is STI | STIs are infections caused by bacteria, viruses, parasites or fungi that can be transmitted from one person to another during unprotected sex, including vaginal, anal or oral sex. | 83.3 |
| | STIs are public health and social problems resulting from the transmission of infectious agents during sexual activity. | 33.3 |
| | STIs are infectious diseases that are transmitted mainly through sexual contact. | 31.3 |
| | STIs are infections caused by poor hygiene of the genitals | 25.0 |
| Mentioned symptoms of STIs | Pain when urinating | 33.3 |
| | Vaginal discharge | 29.2 |
| | Pain during sexual intercourse | 18.8 |
| | Don't know | 16.7 |
| | Itching in the genital area | 2.1 |
| What difference do they make between STIs | No perceived difference | 58.3 |
| | Difference based on pathogen symptoms | 14.6 |
| | Routes of contamination and transmission | 12.5 |
| | Uncertainty or lack of knowledge | 8.3 |
| | Other observations and considerations | 6.3 |

Note: TMP: traditional medicine practitioners; STI: sexually transmitted infections

Regarding differentiation between STIs, 58.3% of the survey participants saw no distinction between the symptoms or other features associated with different STI pathogens, and

additionally, 8.3% indicated lack of knowledge in this respect (table 1). A minority (14.6%) of the participants stated being aware of differences in symptoms caused by different pathogens, yet follow-up discussions yielded ambiguous responses.

For the diagnosis of STIs, 70.8% of the participating TMPs reported relying mainly on symptoms (Table 2). In this context, relying on symptoms refers to the subjective experiences directly reported by the patient. Transmission route was mentioned by 27.1% of the participants as the primary diagnostic criteria, meaning that these TMPs base their diagnostic reasoning on the patient's statement on sexual route of transmission. Consequently, any disease acquired through sexual contact is classified by them as a STI. Only a few (6.3%) participants referred to clinical manifestations or clinical signs as their primary means for STI diagnosis, relating to objective observations gathered by themselves during the patient's appointment. Furthermore, some (12.5%) preferred directing patients to laboratory tests for diagnosis before starting their treatment, while 4.2% reported using traditional methods like fetish consulting.

While TMPs demonstrated overall limited differentiation between STI pathogens, some TMPs mentioned that they specifically recognize HIV infections. They stated that, for the treatment of infections caused by HIV, diagnosis is carried out directly through laboratory analyses in formal healthcare setting.

Since the differentiation between bacterial STI pathogens fell beyond the scope of all participating TMPs, we aimed to gather data more specifically concerning *C. trachomatis* infections by asking the TMPs about their knowledge on trachoma. Trachoma is an eye infection caused by *C. trachomatis*, being a target of WHO-led eradication campaigns yet remaining endemic in some sub-Saharan countries [34]. However, all participating TMPs stated that they are not familiar with the condition and do not have any treatment for trachoma.

**Table 2:** Methods of diagnosing sexually transmitted infections by the participating traditional medicine practitioners

| Topic inquired | Answers by TMPs | Percentage (%) |
|---|---|---|
| Does TMP treat STIs | Yes | 85.4 |
| | No | 14.6 |
| How TMPs differentiate between STIs and other bacterial infections | Specific clinical symptoms | 47.9 |
| | Mode of transmission | 25.0 |
| | Contamination pathways | 27.1 |
| | Symptoms | 70.8 |
| | Laboratory biological diagnostic test | 12.5 |

| | | |
|---|---|---|
| How TMPs diagnose STIs | Clinical manifestations of infection | 6.3 |
| | Traditional consultation | 4.2 |
| | Signs that patients present | 4.2 |
| | Laboratory biological diagnostic test and symptoms | 2.1 |

Note: TMP: traditional medicine practitioners; STI: sexually transmitted infections

### 3.3.Practices of treating sexually transmitted infections

According to the data collected, all participating TMPs used medicinal plants to treat STIs but approximately half (45.8%) of them stated using only medicinal plants for treating STIs. Use of spiritual practices with medicinal plants was the method of choice for 39.6% of the survey participants. Interestingly, this practice was not associate with any specific religious group but was equally applied by animist, Christian and Muslim participants. Additionally, 14.6% reported relying on other practices based on natural mineral or animal substances to complement medicinal plant-based remedies. In most cases (64.6%), TMPs prepared the herbal tea and provided it to the patient without specifying storage conditions or revealing any information about the identity of the used medicinal plant species. The instructions about the storage were given by 29.2% of the participants, while only 6.3% of TMPs stated that they gave storage instructions and also mentioned the names of medicinal plants to the patient.

The participating TMPs expressed mixed views on collaboration with modern medicine in the treatment of STIs. Most (70.8%) stated their treatments should not be combined with modern medicine. The most frequent justifications for this were risks of interaction (27.1%), treatment failure (22.9%), or overdose/toxicity (14.6%). However, 20.8% acknowledged potential complementarity between their herbal preparations and modern medicine. Despite this, 70.8% of practitioners referred patients to hospitals in cases of treatment failure (58.8%) or when unable to treat (41.2%) among the 70.8%. Regarding collaboration among traditional practitioners, 75% referred patients to other TMPs after treatment failure, while 43.8% did so for severe or urgent cases, and 16.7% recommended dual treatments.

### 3.4. Medicinal plants used in the treatment of STIs

During the survey, a total of 63 different medicinal plant species were cited by the participating TMPs for the traditional treatment of STIs (Supplementary Table 3). These plants belong to 37 different botanical families. Plant species cited by at least 10% of TMPs are presented in Table 3. The most frequently mentioned plants were *Ocimum gratissimum*, *Mangifera indica*,

370 *Garcinia kola*, *Xylopia aethiopica*, *Jatropha curcas*, *Jatropha gossypiifolia*, *Syzygium*
371 *aromaticum* and *Phyllanthus amarus*. The number of plant species cited by a single responder
372 varied between 3 and 5 species (average 3.73).
373 According to the statements by the TMPs, the medicinal plants were used both in fresh (54.2%)
374 and dried (45.9%) form. The most often used part of the plants was leaves, followed by fruits,
375 bark and roots (Figure 2A). The TMPs reported preparing medicinal plants as decocted (58.3%),
376 infused (20.8%) and macerated (4.2%) forms (Figure 2B). These preparations were mainly
377 administered orally (93.7%), dermally (4.2%) and rectally (2.1%).
378
379 **Table 3:** Plant species cited by at least 10% of traditional medicine practitioners

| Latin name | Botanical family | Local name | Used parts | Form of use | Relative frequency of citation (%) |
|---|---|---|---|---|---|
| *Ocimum gratissimum* L. | Lamiaceae | Tchiayo | Leaves | Fresh | 37.5 |
| *Mangifera indica L.* | Anacardiaceae | Mangoti | Bark, Leaves | Dry | 25.0 |
| *Xylopia aethiopica (Dunal) A.Rich.* | Annonaceae | Kpéjélékun | Seeds | Dry | 18.7 |
| *Garcinia kola Heckel* | Clusiaceae | Ahowé | Seeds | Dry | 18.7 |
| *Jatropha curcas L.* | Euphorbiaceae | Gbododwi | Leaves | Dry | 16.7 |
| *Jatropha gossypiifolia* L. | Euphorbiaceae | Nyikpotin, gbagidi kpotin | Leaves | Fresh | 14.6 |
| *Syzygium aromaticum (L.) Merr. & L.M. Perry* | Myrtaceae | Atinken Gbadota | Leaves | Fresh | 14.6 |
| *Phyllanthus amarus Schumach. & Thonn.* | Euphorbiaceae | Hlinwé | Leaves | Fresh | 10.4 |

380
381 Regarding the dose used for the treatment of patients suffering from STIs, 35.4% of TMPs
382 stated that the treatment should be used until the clinical signs disappear. Typical

recommendations for treatment duration were between 5–10 days and 10–15 days (Figure 2C). Most TMPs also reported that their medications are used two or three times a day (Figure 2D).

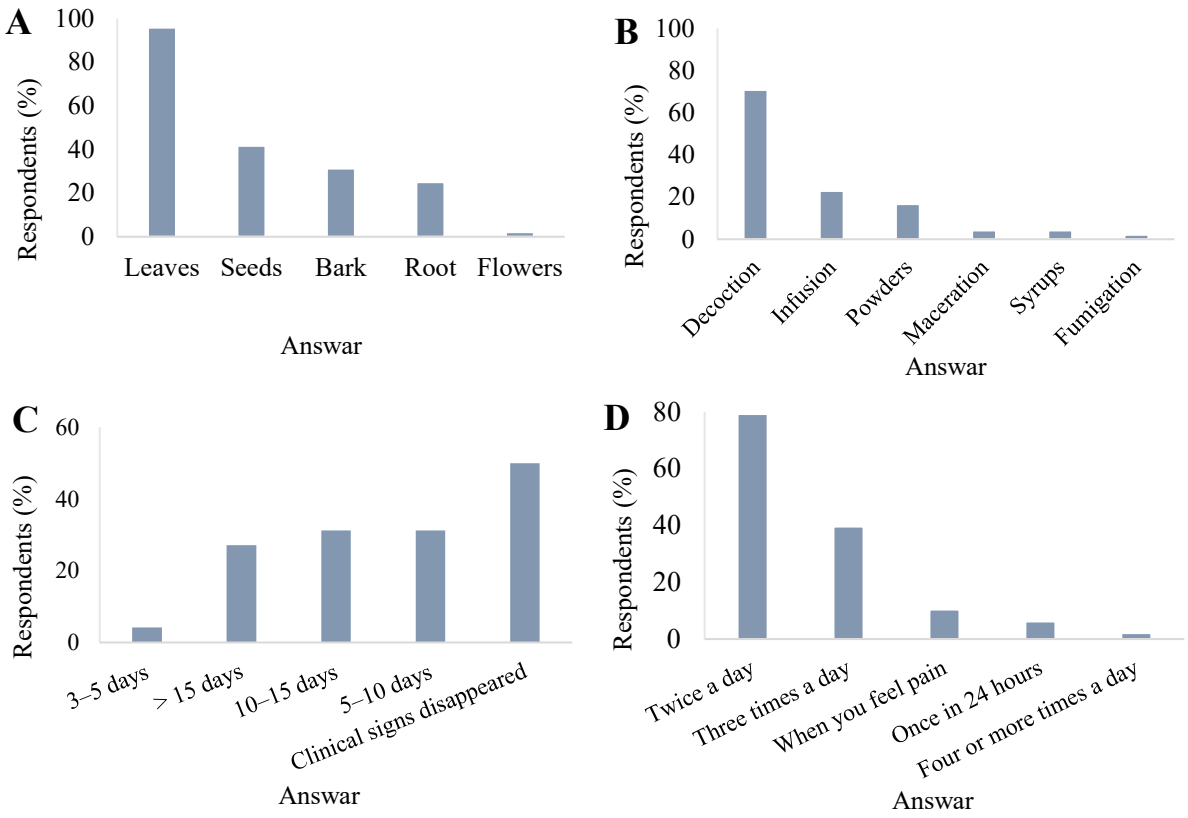



**Figure 2.** Preparation and dosage practices of medicinal plant preparations reported by the traditional medicine practitioners (TMPs) for the treatment of sexually transmitted infections (STIs). A) Plant organs used in the treatment of STIs, B) Form of preparation of medicinal recipes for treatment, C) administration times of therapeutic formula, D) Used dosage for the treatment. Results are presented as percentages of mentions.

Table 4 presents the indicators used by the TMPs to assess treatment efficacy. The results indicate that 31.2% rely on the disappearance of symptoms and clinical signs, 31.2% on patient feedback, and 20.8% on monitoring treatment adaptation. Additionally, 25% conduct patient follow-up and monitoring, while 16.7% make use of laboratory results to confirm treatment effectiveness. Regarding side effects, 93.7% of TMPs reported that their medication does not have adverse effects. However, 10.4% noted that their formulations stimulate appetite, and 4.2% mentioned occasional side effects such as diarrhea, nausea, and headaches.

**Table 4:** Efficacy indicators and treatment-associated side effects mentioned by the Traditional medicine practitioners.

| Topic inquired | Answers by TMPs | Percentage (%) |
|---|---|---|
| Cited indicators of the effectiveness of treatment | Patient feedback and testimonials | 31.2 |
| | Disappearance of symptoms and clinical signs | 31.2 |
| | Monitoring and adaptation of treatment | 25.0 |
| | Patient Follow-up and Monitoring | 20.8 |
| | Diagnostic confirmation and medical analyses | 16.7 |
| | Disappearance of symptoms, patient testimonies | 4.7 |
| Cited side effects of the treatment | None | 93.7 |
| | Stimulates appetite | 10.4 |
| | Diarrhea, nausea and headache | 4.7 |
| | Fatigue | 2.1 |

Note: TMP: traditional medicine practitioners; STI: sexually transmitted infections

### 3.5. Impact of selected plant extracts on epithelial cell viability

To experimentally evaluate the pharmacological basis of the traditional medicine preparations, four plant species were selected based on their RFC within the TMP interview. Despite being frequently cited by TMPs, we excluded *Garcinia kola* from the experimental study as this species is extinct in Benin and several neighboring countries due to overharvesting [35].

Since our *in vitro* efficacy studies were focused on the obligately intracellular pathogen *C. trachomatis*, the experimental study was initiated by evaluating host cell viability upon exposure to the plant extracts to exclude false positive results. The cytotoxicity effect of prepared plant extracts was studied in Hela-229 cells for 48 hours using concentrations ranging from 5 µg/ml to 250 µg/ml. For ethanolic extracts, decreased HeLa-229 cell viability was observed at the highest concentrations with *O. gratissimum* leaves and *P. amarus* leaves. Neither *M. indica* (bark and leaves) nor *J. curcas* (leaves) showed cytotoxic effects *in vitro* in any used concentration (Supplementary Figure 1A). No cytotoxicity effect was noticed with aqueous extracts with any used plant material (Supplementary Figure 1B). $CC_{50}$ values of each plant extract are presented in Table 5. The concentrations of each plant extract for anti-chlamydial assays were chosen based on cytotoxicity results, excluding the cytotoxic concentrations in each case.

419

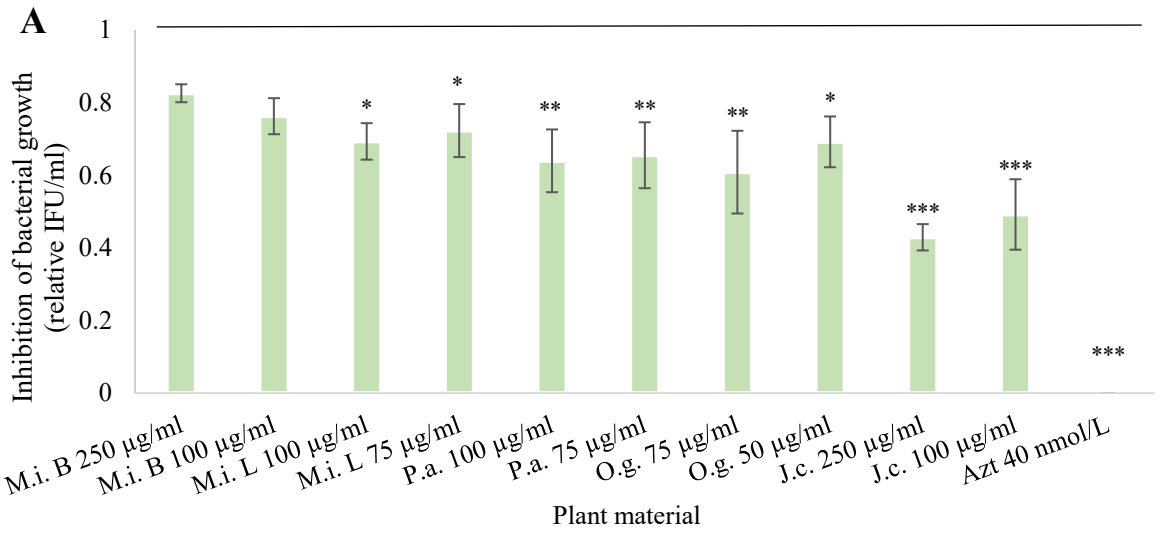
420 **Table 5.** $CC_{50}$ values of selected plant extracts

| Plant species and plant part | Extract | $CC_{50}$ | $R^2$ |
|---|---|---|---|
| ***Mangifera indica* bark** | Ethanolic extract | 29.9 | 0.55 |
| | Aqueous extract | 60.3 | 0.95 |
| ***Mangifera indica* leaves** | Ethanolic extract | 146.4 | 0.63 |
| | Aqueous extract | 90.7 | 0.78 |
| ***Phyllanthus amarus* leaves** | Ethanolic extract | 173.9 | 0.86 |
| | Aqueous extract | 100.1 | 0.94 |
| ***Ocimum gratissimum* leaves** | Ethanolic extract | 99.5 | 0.98 |
| | Aqueous extract | 34.2 | 0.37 |
| ***Jatropha curcas* leaves** | Ethanolic extract | 22.0 | 0.62 |
| | Aqueous extract | 83.2 | 0.97 |

421 **3.6. Anti-chlamydial effects of the plant extracts**

422 **3.6.1. Inhibition of chlamydial growth**

423 To investigate the impact of the plant extracts against growth of the pathogenic strain K of
424 *Chlamydia trachomatis*, ethanolic and aqueous extracts of all five chosen plant materials were
425 assayed for their ability to reduce chlamydial inclusion counts. With ethanolic extracts, the
426 highest inhibition was observed with *J. curcas* 250 µg/ml ($P = 0.001$) and 100 µg/ml ($P = 0.002$)
427 (Figure 3 A). A slightly lower but significant effect was observed for *M. indica* leaves 100 µg/ml
428 ($P = 0.021$) and 75 µg/ml ($P = 0.045$), for *O. gratissimum* 75 µg/ml ($P = 0.002$) and 50 µg/ml
429 ($P = 0.021$) and for *P. amarus* 100 µg/ml ($P = 0.005$) and 75 µg/ml ($P = 0.008$). In contrast, no
430 significant suppression of chlamydial growth was observed with the ethanolic extract from the
431 bark of *M. indica*.



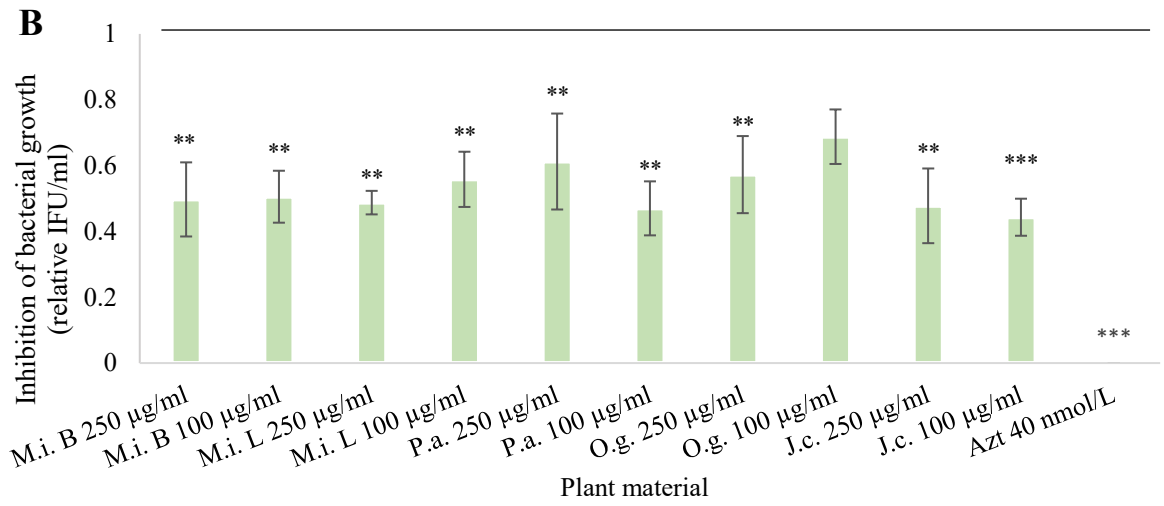




**Figure 3.** Inhibition of *C. trachomatis* growth by the medicinal plant extracts. The impact of ethanolic (A) and aqueous (B) extracts on *C. trachomatis* inclusion counts in HeLa-229 cells were quantified with immunofluorescence staining after 48 h infection. Results are presented as IFU/ml values normalized to the DMSO treated infection control and shown as mean ± SEM ($n = 4$). Significance is presented as marks of *P*-values compared to infection control, *P*-values: < 0.05: *; < 0.01: **; < 0.001: ***. $\eta^2$ was 0.799 for ethanolic extracts and 0.697 for aqueous extracts. Abbreviations: M.i: *Mangifera indica*, P.a.: *Phyllanthus amarus*, O.g.: *Ocimum gratissimum*, J.c.: *Jatropha curcas,* Azt: azithromycin, L: leaf, B: bark.

Regarding the aqueous extracts, the analyses reveal that apart from *O. gratissimum* 100 µg/ml, for which no significant difference was noted, all other medicinal plant extracts showed significant effect compared to the nontreated control group. Consistent with the data obtained

with ethanolic extracts, *J. curcas* demonstrated highest *C. trachomatis* growth inhibition also as an aqueous extract. The *P*-values range from 0.001 to 0.009 (Figure 3 B).

Because the highest inhibition of *C. trachomatis* growth was observed with ethanolic extracts in a concentration dependent manner, the highest concentration of each ethanolic extract was chosen to determine inhibition of bacterial genome replication by qPCR. Significant decrease of genomic copy numbers of *C. trachomatis* was observed with *M. indica* bark*, P. amarus, O. gratissimum* and *J. curcas* extracts, consistent with the results on chlamydial inclusion counts determined by immunofluorescence staining. Data are presented in Figure 4. Ability of the ethanolic extracts to reduce chlamydial genome copy numbers was also confirmed in the absence of CHX to rule out its interference with the extracts. This control experiment yielded inhibition percentages comparable to those obtained in the presence of CHX (data not shown), hence sorting out such potential interfering factor.

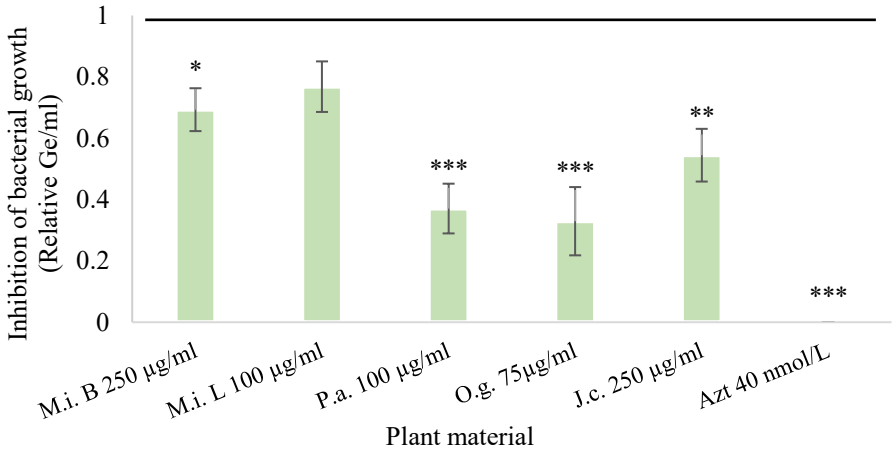



**Figure 4:** Inhibition of *C. trachomatis* genomic replication by the ethanolic extracts of the studied medicinal plants. *C. trachomatis* genome copy numbers were determined with qPCR after 48 h infection in HeLa-229 cells. Results are presented as genome equivalents (GE/ml) values normalized to the DMSO treated infection control and shown as mean ± SEM ($n$ = 4). Significance is presented as marks of *P*-values compared to infection control, *P*-values: < 0.05: *; < 0.01: **; < 0.001: ***. $\eta^2$ was 0.775. Abbreviations: M.i: *Mangifera indica*, P.a.: *Phyllanthus amarus*, O.g.: *Ocimum gratissimum*, J.c.: *Jatropha curcas,* Azt: azithromycin, L: leaf, B: bark.

### 3.6.2. Inhibition of Chlamydial infectious progeny production

The inhibition of chlamydial infectious progeny production was studied as described above with all five plant materials both as ethanolic and aqueous extracts. The results revealed a

reduction in bacterial load compared to the control in all tested concentrations of the ethanolic extracts from all medicinal plants. The *P*-values range from 0.001 to 0.031 (Figure 5 A).

Regarding the aqueous extracts, a weak effect was noted with all plant extracts, but only *P. amarus* leaves 250 µg/ml ($P = 0.006$) and 100 µg/ml ($P = 0.016$), *M. indica* bark 250 µg/ml ($P = 0.021$) and *O. gratissimum* leaves 250 µg/ml ($P = 0.043$) were statistically significant compared to the vehicle control (Figure 5 B).

Ethanolic extract showed more potent effect to inhibit chlamydial progeny production, where *P. amarus* and *O. gratissimum* were most effective, as those were effective also in inhibition of growth.

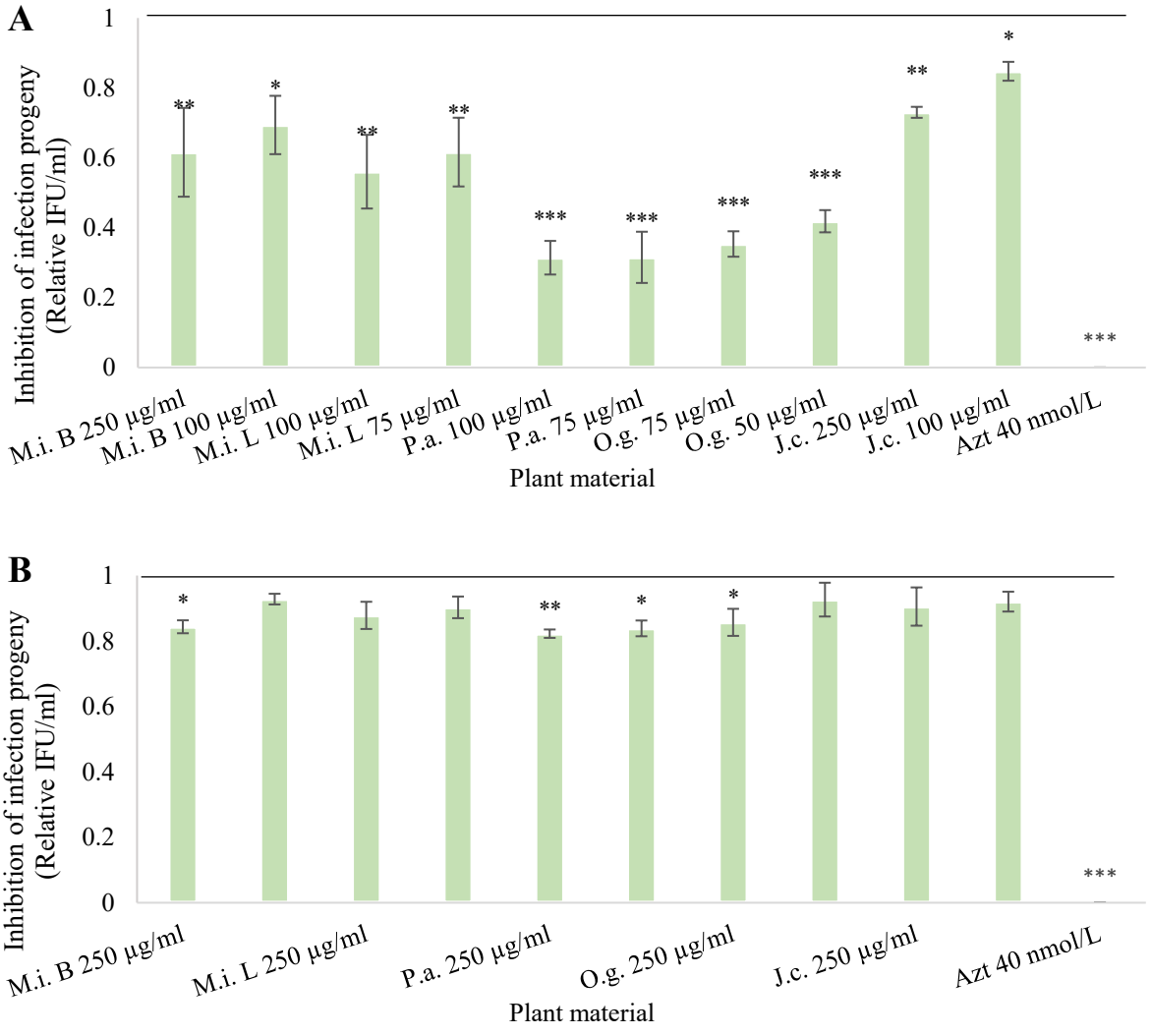



**Figure 5.** Inhibition of *C. trachomatis* infectious progeny production by the studied medicinal plant extracts. The impact of ethanolic (A) and aqueous (B) extracts were determined by treating infected HeLa-229 cells with the extracts for 48 h, lyzing the cells and inoculating the lysates to fresh HeLa-229 cell monolayers. Chlamydial inclusion count was determined with

immunofluorescence at 48 h post-inoculation. Results are presented as IFU/ml values normalized to the DMSO treated infection control and shown as mean ± SEM ($n = 4$). Statistical significance is presented as marks of *P*-values compared to infection control, *P*-values: < 0.05: *; < 0.01: **; <0.001: ***. $\eta^2$ was 0.851 for ethanolic extracts and 0.942 for aqueous extracts. Abbreviations: M.i: *Mangifera indica*, P.a.: *Phyllanthus amarus*, O.g.: *Ocimum gratissimum*, J.c.: *Jatropha curcas,* Azt: azithromycin, L: leaf, B:bark.

### 3.7 Impact on *C. trachomatis* EB infectivity

To evaluate the ability of the plant extracts to reduce the infectivity of chlamydial extracellular, infectious EB forms, *C. trachomatis* EBs were exposed to the ethanolic extract of each plant material at its highest non-cytotoxic concentration for 1 h and subsequently used to infect HeLa-229 cells. All tested extracts, except *J. curcas*, reduced the EB infectivity rate. In contrast to assays targeting chlamydial intracellular growth stage, significant effect of inhibition was observed with *M. indica* extracts (bark and leaves). Data are presented in Figure 6.

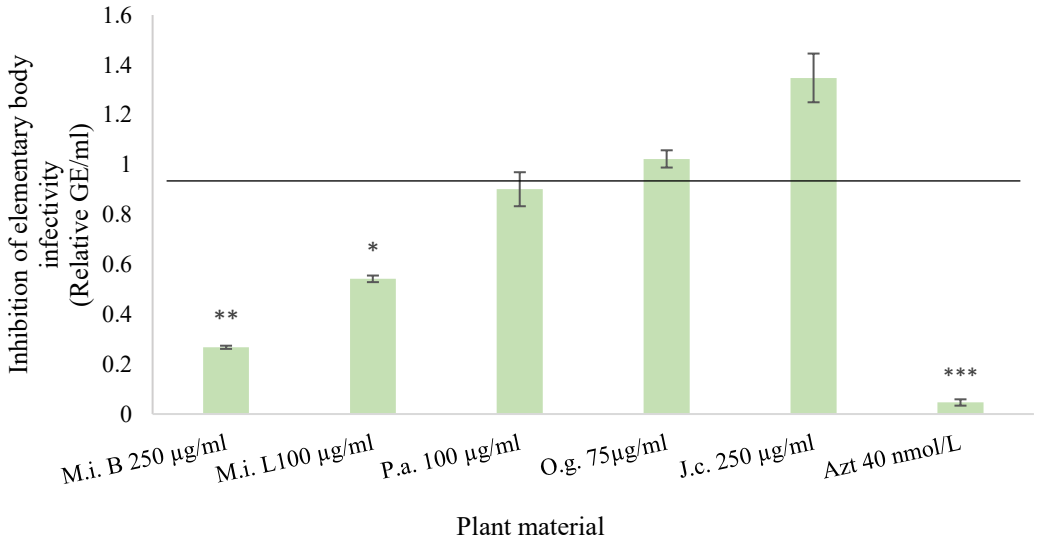



**Figure 6.** Suppression of *C. trachomatis* EB infectivity by the ethanolic plant extracts. EB suspensions were exposed to the extracts for 1 h prior to inoculation to HeLa-229 cells and quantifying *C. trachomatis* genome copy numbers in the cell lysates 24 h post-infection. Results are presented as genome equivalents (GE/ml) values normalized to the DMSO treated infection control and shown as mean ± SEM ($n = 4$). Significance is presented as marks of *P*-values compared to infection control, *P*-values: <0.05: *; < 0.01: **; < 0.001: ***. $\eta^2$ was 0,962.

Abbreviations: M.i = *Mangifera indica*, P.a. = *Phyllanthus amarus*, O.g. = *Ocimum gratissimum*, J.c. = *Jatropha curcas,* Azt = azithromycin, L = leaf, B = bark.

## 4. Discussion

Traditional medicine maintains its central role in managing chronic diseases and infections in regions with limited access to modern healthcare. As highlighted also in the WHO traditional medicine strategy, effective measures are needed to promote the evidence-based and standardized exploitation of this knowledge, to prevent its loss and to promote its integration into formal health systems to advance access to health in a culturally sensitive fashion [36]. The current study contributes to this goal by exploring the traditional management of STIs in the west African country Benin, accomplished through the combination of an interview study among Beninese TMPs and experimental evaluation of the collected plant material.

A generally acknowledged challenge in addressing traditional medicine through scientific research is the lack of mutual trust between TMPs, academics and representatives of formal healthcare systems. The relatively small number of participating TMPs in the current study reflects to some extent this phenomenon and must be considered a limitation for our work. Furthermore, the interviewed TMP group is very likely biased to involve the practitioners with positive attitudes towards academic collaboration. Results of the interview study hence cannot be considered a fully representative picture of the knowledge, attitudes and practices of the whole traditional medicine sector in the country. However, we did manage to engage a geographically, ethnically and linguistically diverse set of participants, in which also the three main religions of the country were equally represented. Consistent with the overall gender distribution among Beninese TMPs, majority of the participating practitioners in our study were male. While this highlights the socio-cultural norms of traditional knowledge transmission, the lower participation of women may affect care dynamics. Considering the high prevalence of STIs among females in sub-Saharan Africa, the need to address cultural barriers and encourage female inclusion must be emphasized in all future initiatives on STI management.

Our results indicate widespread awareness of STIs among TMPs in Benin. However, the lack of differentiation between different STIs among virtually all participating practitioners is alarming from the patient safety perspective, as accurate identification of the type of STI would be crucial to administer appropriate treatment and prevent the spread of infections. This likely attributes to insufficient training and lack of educational resources: TMPs practice their profession without formal education and typically have limited access to continuing education

or up-to-date information on infectious diseases. In fact, despite its persistent dominance in primary health management, no formal education in phytomedicine has been available in the country before the pilot training program launched in autumn 2025 (https://minnagan.com).

Analysis of the diagnostic methodologies for STIs by Beninese TMPs revealed that the practice heavily relies on patient-reported symptoms and route of transmission. However, most such symptoms are not specific to STIs, as also conditions like vaginal candidiasis can present with similar clinical manifestations. This practice can lead to diagnostic ambiguity, inappropriate treatments, and significant medical and social consequences. The limited use of laboratory diagnostic tests in the TMPs' practice is not surprising, considering the fact that Beninese traditional medicine sector remains isolated from the formal health care system. In practice, TMPs have laboratory diagnostic information available only if the patient themselves takes the initiative to request testing in a local health center and then share the test result with their TMP. The reported use of traditional fetish consultation, although in the minority, underlines the sustained spiritual dimension of traditional medical practice. Despite being often dismissed or ignored by researchers and public authorities, it represents an integral component of the holistic model of health that TMPs apply and should be understood on its own epistemic terms.

Reliance on clinical symptoms for STI diagnosis can impact the accuracy and effectiveness of treatment. *C. trachomatis*, for example, is often asymptomatic or has symptoms similar to other STIs, making it difficult to accurately identify without specific laboratory testing [37], This diagnostic challenge is not unique to traditional practice but remains a well-documented obstacle in formal clinical settings as well [38]. Collectively, these findings highlight the need to strengthen the diagnostic capacities of TMPs and to promote collaboration with modern medicine, particularly through referral for laboratory testing whenever possible. A few TMPs in our survey specifically mentioned treating HIV in patients who had first obtained a laboratory diagnosis for this condition, which further illustrates the complexity of the ethical and patient safety concerns involved at the interface of traditional and formal health systems.

Since our study was designed to target *C. trachomatis* for the experimental evaluation part, our interview study included also a section concerning the practitioners' knowledge on trachoma and the medicinal plants they use for its treatment. Trachoma is an eye disease caused specifically by certain *C. trachomatis* strains, remaining endemic in some sub-Saharan and other low-income countries despite extensive eradication campaigns. The practitioners reported that they were not familiar with this condition, and in fact, many of them confused trachoma with conjunctivitis. As officially announced by the Beninese Minister of Health on the Ministry's official website, trachoma has been eradicated as a public health problem in Benin

574 in 2021. Although most TMPs participating in our survey had at least 10 years of experience in
575 their practice, none of them recognize trachoma. This specific example illustrates typical
576 semantic challenges in ethnomedicine, highlighting the importance of accurate diagnosis when
577 interpreting ethnopharmacological data.
578 As for the duration of treatment, the variability in responses from traditional practitioners
579 illustrates a lack of consensus or standardization in treatment protocols. Moreover, it reflects a
580 deep grounding in practices based on clinical observation and feedback, rather than rigorous
581 standardization. The fact that only 12.5% of TMPs use laboratory results to validate the
582 treatment effectiveness demonstrates an important gap in the integration of modern diagnostic
583 methods into traditional medicine. It is also noteworthy that the majority of TMPs reported the
584 absence of side effects. This observation is common in traditional medicine practices, where
585 side effects are often less documented due to a lack of systematic follow-up [39].
586 The Beninese TMPs mainly resort to the use of medicinal plants for treating STIs, but in parallel
587 to them, many TMPs apply spiritual practices. While spiritual aspects exist as integral part of
588 the ontology of health and disease in the region, the significance of the spiritual elements in
589 TMP practice may also partially reflect the needs for maintaining exclusivity in the absence of
590 intellectual property frameworks. Indeed, protection of traditional knowledge, involving the
591 ownership of both traditional medicine recipes and local biodiversity resources is a key question
592 to consider in any attempts to integrate traditional medicine to formal health systems. Such
593 ethical concerns require balancing with the need for transparency, documentation and
594 standardized practices for the effective and evidence-based use of the plant-based treatments.
595 In the current study, protection of traditional knowledge was considered through the decision
596 to report only individual plant species and describe preparation methods and treatment practices
597 in general level, whereas actual traditional medicine recipes involving plant species
598 combinations and specific preparation methods are not included in this manuscript.
599 Our results show a considerable diversity of medicinal plants used to treat STIs in traditional
600 practices. As exemplified by the case of *Garcinia kola* in this survey, widely utilized medicinal
601 plants include species with endangered or even extinct status. Sustainable use of local
602 biodiversity for healthcare purposes hence needs to integrate with conservation science and
603 close involvement of local TMPs. In fact, our recent findings indicate that while Beninese TMPs
604 are generally aware of the endangered status of local plants, the specific plant species which
605 they list in this context have low correlation with official records [40].
606 Our cytotoxicity assays on the selected plant extracts indicate that the aqueous extracts of all
607 studied materials showed no detectable cytotoxicity in HeLa-229 cells after 48 hours of

exposure, while ethanolic extracts from *Mangifera indica* (bark and leaves) and *Jatropha curcas* leaves were also found not to show acute cytotoxicity at the tested concentrations. The absence of toxicity at relevant concentrations indicates that these plant extracts may be reliably used for subsequent *in vitro* antimicrobial assays. This provides a solid basis for future investigations aiming to validate their therapeutic potential against *C. trachomatis* and to identify bioactive compounds responsible for the antichlamydial activity. From clinical perspective, more extensive safety and toxicity profiling is naturally required to validate their safe use in humans.

According to our results, all 5 top-cited plant materials selected for experimental evaluation showed *in vitro* antichlamydial activity. In particular, the ethanolic extracts of *P. amarus*, *O. gratissimum* and *J. curcas* exhibited significant inhibition of *C. trachomatis* growth, genomic replication and infectious progeny production. The antichlamydial effect of the extracts was dose-dependent and the potency of ethanolic extracts was found to be superior compared to their aqueous counterparts. This suggests that the antichlamydial compounds in these plants are more efficiently solubilized in ethanol than in water. This observation aligns with the well-established principle that solvents with intermediate polarity, like ethanol, are highly effective in extracting a broad spectrum of antimicrobial secondary metabolites, including polyphenols, alkaloids, and terpenoids. Disseminating these data and their practical implications to TMPs relate to the need of standardized preparation and administration protocols for the used plant material. These findings are consistent with, and add to, a small but growing body of literature demonstrating that plant-derived extracts can inhibit *C. trachomatis in vitro*. The study from Entrocassi et al. [41], with Argentinian ethnomedicinal plants, found that a dichloromethane extract of *Hydrocotyle bonariensis* broadly inhibited the exponential growth stage of both *C. trachomatis* and *C. pneumoniae* across multiple clinical genotypes.

Interestingly, in contrast to intracellular bacterial growth assays*, P. amarus*, *O. gratissimum* and *J. curcas* extracts did not show any impact on the infectivity of *C. trachomatis* EBs, while *M. indica* was more potent than other extracts in this respect. The data on *M. indica* may indicate an ability to disrupt infective form of *C. trachomatis*, whereas compounds from other studied extracts are more effective against the replicative and metabolically active phenotype of the bacteria. These findings illustrate the unique biology of *Chlamydia* spp. bacteria, with two phenotypes showing distinct morphology and metabolism. Together with the obligate intracellular growth style, the biphasic infection cycle poses specific demands for experimental antichlamydial studies in resource-limited settings. In fact, to the best of our knowledge this

study represents the first published report on the antichlamydial activity of medicinal plants from sub-Saharan Africa.

The lack of phytochemical composition data on the studied plant material represents a key limitation of the current study. However, previous research has addressed the bioactive metabolites in all four studied plants [42–45], and future research is to be directed on detailed understanding of the role of individual metabolites in the antichlamydial activity and to develop the plant-derived extracts to standardized herbal medicines based on this information. In addition, *M. indica* leaves have been repeatedly shown to be rich in gallotannins, mangiferin (a C-glucosylxanthone), flavonoids (quercetin and kaempferol derivatives) and phenolic acids, several of which possess documented membrane-disrupting and antibacterial properties [46]. *P. amarus*, as reviewed comprehensively across the genus by Mao et al. [47], is characterized by more than 510 isolated compounds dominated by lignans (notably phyllanthin and hypophyllanthin), triterpenoids, flavonoids and tannins, several of which underlie its well-documented antiviral activity and would be plausible candidates for the antichlamydial activity observed. *O. gratissimum* leaves are dominated by flavonoids and phenolic acids, both recognized antimicrobial metabolite classes [45]. *Jatropha* species contain flavonoids, saponins, tannins, alkaloids and terpenoids in varying proportions across plant organs and comparative extraction-method studies confirm that the biological activity of *Jatropha* species extracts varies considerably depending on plant part and solvent polarity [48].

Earlier studies have reported antibacterial activity of *O. gratissimum* [49], *M. indica* [50], *P. amarus* [51–53] and *J. curcas* [54] against other bacterial species. The extract concentrations used in our study are in line with previous literature and may be achievable in clinical use. Overall, our *in vitro* chlamydial susceptibility results encourage further investigation of the frequently cited plant species against STI pathogens. Significant knowledge gaps remain concerning the chemical composition of the extracts, oral bioavailability and other pharmacokinetic properties of the pharmacologically active constituents.

This study focused on evaluating plant extracts against chlamydia infection due to its high prevalence. Given the symptom-oriented usage practices evident by the collected ethnopharmacological data, it would be appropriate to expand the experimental evaluation to other sexually transmitted microbes. Future research should also involve *in vivo* animal models, pharmacokinetic studies and controlled clinical trials. To build on these findings, an integrated research approach is required, calling for capacity building and improved technical infrastructures to advance scientific rigor. Work towards integrating traditional medicine and biodiversity resources into health systems is essential to promote contextually relevant means

for socioeconomic development in sub-Saharan Africa. To this end, developing, in collaboration with practitioners and policymakers, frameworks for cooperation and more standardized use of medicinal plants can offer a path forward.

## Conclusions

Building on the call for closer, ethically grounded collaboration between traditional and formal health systems, the current study shows that STI management in Benin involves rich cultural diversity yet holds potential for evidence-based phytomedicine. Our TMP survey revealed a wide diversity of practices among the practitioners, alongside gaps in STI differentiation and diagnosis where collaboration with formal healthcare could add value. Experimental evaluation of the most cited plants confirmed measurable *in vitro* antichlamydial activity for all, with ethanolic extracts consistently more potent and *M. indica* uniquely targeting the infective elementary body form. Together, these findings substantiate the argument that traditional knowledge held by Beninese TMPs is a viable starting point for evidence-based phytomedicine, capable of guiding antichlamydial compound discovery and contributing to the fight against antimicrobial resistance. Realizing this potential requires integrating traditional medicine into local health systems through equitable, evidence-based frameworks that respect knowledge holders, including access and benefit-sharing mechanisms, to deliver accessible, culturally relevant STI therapeutic options for West Africa.

## List of abbreviations

CHX = cycloheximide, EB = elementary body, HIV = human immunodeficiency virus, MOI = multiplicity of infection, RB = reticulate body, RFC = relative frequency of citation, STI = sexually transmitted infection, TMP = Traditional Medicine Practitioner, WHO = World Health Organization

## Declarations

### Ethics approval and consent to participate

This study was conducted in full compliance with United Nations Convention of Biological Diversity and the Nagoya protocol. The interview survey was approved by the National Pharmacopoeia and Traditional Medicine Program office, which serves as the official regulatory body for traditional medicine practitioners in Benin. The study was also approved by the Ethics Committee of the Research Unit in Applied Microbiology and Pharmacology of

natural substances at the University of Abomey-Calavi in Benin (approval no. 0018/2024/CE/URMAPha/UAC). The traditional healers included in this study were identified and recruited through the office. Prior to their participation, each healer provided verbal informed consent after receiving detailed explanations about the study. Participation was fully voluntary and the practitioners did not receive any compensation. The plant materials used are not classified as endangered.

Clinical trial number: Not applicable

### Consent for publication

All authors gave their consent for publication

### Data availability

All data generated from this study are included in the manuscript, tables, figures and supplementary file. The questionnaire of the survey is also available.

### Competing interests

The authors declare that no conflicts of interest exist regarding the submitted manuscript.


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### Author's contributions

**Leena Hanski:** Conceptualization, Review and editing of manuscript, Validation, Supervision, Methodology, Resources, Project administration, Funding acquisition

**Phenix Assogba**: Writing original draft, review and editing, Visualization, Methodology, Investigation, Formal analysis

**Eveliina Taavitsainen-Wahlroos**: Writing original draft, review and editing, Visualization Validation, Supervision, Methodology, Investigation, Formal analysis

**Victorien Dougnon**: Methodology, project administration, resources, supervision, review and editing

**Boris Legba**: methodology, investigation, formal analysis, review and editing

**Sophonia Ahouandjinou** : investigation, formal analysis, review and editing

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