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RESEARCH ARTICLES

Deciphering the antimycobacterial, cytotoxicity and phytochemical profile of *Entada abyssinica* stem bark

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Abstract
Organic (acetonic, chloroform, methanolic and hexanic) extracts of *Entada abyssinica* stem bark were screened for their antimycobacterial, cytotoxicity and phytochemical profiles. Microplate Alamar Blue and MTT assays were used to establish the minimum inhibitory concentration (MIC) of the extracts against *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*, and median cytotoxic concentrations (CC_{50}) against Vero E6 cells, respectively. The most bioactive extracts were subjected to phytochemical screening, FTIR and GC/MS analyses. Results obtained showed that acetonic and methanolic extracts were the most bioactive (MIC range: 125 to 468 µg/mL) while the CC_{50} of all the extracts were greater than 500 µg/mL. GC/MS analysis revealed 14 compounds in the acetonic and methanolic extracts and these were mainly esters. Of these, a known antimycobacterial compound (oleic acid) was identified. We conclude that acetonic and methanolic extracts of *Entada abyssinica* stem bark possess promising antimycobacterial activity, indicating the need to isolate pure compounds and test them in an effort to unveil novel and more effective antitubercular drugs.

Keywords *Entada abyssinica* · Tuberculosis · Traditional medicine · Medicinal plants · Drug discovery

Introduction

Tuberculosis is an infectious respiratory disease whose causative microbe is *Mycobacterium tuberculosis* (Rodriguez-Takeuchi et al. 2019). It continues to be a disease of public health importance due to its co-infection with HIV, high incidence as well as mortality rates (WHO 2021). In 2020, more than 1 million global deaths were due to tuberculosis (TB), with Africa and South Eastern Asia contributing upto 80% of the deaths. The East African Community is among the top countries facing the brunt of TB (WHO 2021). Despite the strides made to realize effective TB treatment regimens, the rapidly emerging multidrug-resistance in *Mycobacterium tuberculosis* (*Mtb*) have resulted into unsuccessful treatment outcomes (Falzon et al. 2017). Additionally, the available drugs also possess adverse reactions as well as interactions with antiretroviral drugs (Ambrosio et al. 2015).

In consequence, the use of medicinal plant preparations for managing TB has become common among indigenous communities because they are more accessible, affordable and perceived to be safe (Obakiro et al. 2020). However, the utilization of medicinal plants for TB treatment is criticized because of insufficient scientific evidence that supports the claimed efficacy and safety as well as absence of established standard procedures for the preparation, storage, dose administration and patient monitoring communities.

Entada abyssinica A. Rich (Fabaceae) is a deciduous tree of Eastern and Central Africa. Across East and West Africa,

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its stem and root barks, roots and leaves are ingredients of herbal medicament for TB, asthma, eye inflammation, bronchitis, rheumatic fever, diabetes, snake envenomation, erectile dysfunction, sleeping sickness, abdominal and arthritic pains (Freiburghaus et al. 1998; Kyarimpa et al. 2023; Obakiro et al. 2020; Nyasse et al. 2004; Sobeh et al. 2020). Although most of its bioactivities have been investigated (Dzoyem et al. 2017), the antimycobacterial activity of *E. abyssinica* has only been validated for its leaf extracts against *M. tuberculosis* and *M. smegmatis* (Mariita et al. 2010). The stem bark, which is the most used part of this plant around the Lake Victoria basin of East Africa, has not been investigated. This contribution is an attempt to decipher the antimycobacterial potential, cytotoxicity and bioactive phytochemicals of *E. abyssinica* stem bark.

Methods

Stem bark sampling and extraction procedures

Stem bark of *E. abyssinica* were sampled from Siaya and Kisumu counties of Kenya in January 2020. Voucher specimen no. OSB/01/2020/001 has been deposited at the University of Eldoret Herbarium, Kenya. The obtained stem barks were pooled and taken to Chemistry laboratory, Moi University, Kenya. The barks were chopped and dried at room temperature for 1 month. The samples were then pulverized and stored in clean labeled paper bags.

Phytochemicals were serially extracted from 300 g of stem bark powder by maceration using 1000 mL of hexane, chloroform, acetone, and methanol for 72 h. The crude extracts were obtained through filtration of the crude mixtures through cotton wool and Whatman filter paper, followed by concentration on a rotary evaporator. The extracts obtained were dried using anhydrous copper (II) sulfate and the extraction yields were thereafter computed for each solvent.

Antimycobacterial testing

Mycobacteria used in this study namely: *M. smegmatis* (ATCC607) and *Mtb* ($H37R_v$) were provided by Kenya Medical Research Institute, Kenya. They were prepared along with the extracts for antimycobacterial assay as detailed by Obakiro et al. (2022). Briefly, Microplate Alamar Blue assay was used to determine the minimum inhibitory concentration (MIC) of the extracts in a 96-well plate using rifampicin as the positive control (standard drug). MIC was defined as the lowest concentration of the extract which prevented color change from blue to pink (Obakiro et al. 2022).

Assessment of cytotoxicity of the extracts

The MTT assay was employed for assessment of potential cytotoxicity of the extracts against normal Vero E6 cells (ATCC CCL 81) following standard procedures (Mosmann 1983) detailed in our previous report (Obakiro et al. 2022).

Bioassay-guided phytochemical studies of acetonic and methanolic extracts

Phytochemical screening procedures

Classical phytochemical screening of the bioactive extracts was done to establish the presence of triterpenes, saponins, tannins, flavones, steroid glycosides, coumarins, anthocyanins, volatile oils, reducing sugars and alkaloids following standard procedures (Koparde 2017).

Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FTIR) spectra of the acetonic and methanolic extracts were obtained on a JASCO FTIR-6600 spectrometer (Jasco, Japan). The scans were run between 4000 and 400 cm^{-1} using 1 mg of the solid extracts at a spectral resolution of 4 cm^{-1}.

Gas chromatography-mass spectrometry analysis

Acetonic and methanolic extracts (5 mg) were redissolved in 5 mL of their respective extraction solvents. Phytochemicals in the extracts were derivatized using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) following the manufacturer's instructions.

The GC-MS analysis was performed on an Agilent Intuvo 9000 GC system (Agilent Technologies, USA) equipped with a fused silica column (Agilent Intuvo HP-5MS UI) with dimensions: 30 m × 250 µm × 0.25 µm. The MS component was Agilent 7000D GC/TQ. The exact conditions are described elsewhere (Obakiro et al. 2022). Compound identification was done by comparison of retention times and mass spectra with compounds in the NIST library and published spectroscopic data.

Data analysis

All experiments were replicated once and the obtained results were presented as means ± standard deviations of replicates. Assessment of statistically significant differences among means was achieved through performing One Way ANOVA in GraphPad Prism (v9, GraphPad software,

USA). Means were considered to be significantly different at $P < 0.01$ if not $P < 0.05$.

Results and discussion

Extraction yield of*E. abyssinica*stem bark extracts

Methanolic extract had the highest yield (9.1%) followed by acetone (5.9%), chloroform (1.13%), and then hexane (0.66%). The yields increased with increasing solvent polarity, with methanol extracting the highest amount of the phytochemicals. Since polar solutes dissolve in polar solvents and vice versa, these results indicate that *E. abyssinica* stem bark is rich in polar phytochemicals. A previous report (Chitopoa et al. 2019) indicated that methanolic extract of *E. abyssinica* leaves had a better yield (15%), than hexane (0.18%), dichloromethane (0.07%) and ethyl acetate (0.01%) solvent extracts.

Antimycobacterial activity and cytotoxicity of*E. abyssinica*stem bark extracts

Against *M. smegmatis*, acetonic and methanolic extracts had the highest antimycobacterial activities (MIC = 1250 ± 0.0 µg/mL in both cases) while chloroform and hexane extracts had the lowest activities (MIC = 2500 ± 0.0 µg/mL). When tested against *Mtb*, methanolic extract had better bioactivity (MIC = 468 ± 22 µg/mL) compared to the acetonic extract (MIC = 937 ± 442 µg/mL) (Table 1). Hexane and chloroform extracts, however, showed no appreciable activity against *Mtb*. This diminished bioactivity maybe due to the fact these solvents extract largely non-polar compounds which may not be bioactive (Abuzeid et al. 2014). This is also supported by the weak antimycobacterial activity of these extracts against *M. smegmatis*. On the other hand, the higher antimycobacterial activity of methanol extract than the acetone extract is because the former is more polar, and thus extracts more polar phytochemicals. In line with our results, the methanolic leaf extract of *E. abyssinica* was previously reported to possess antimycobacterial activity against *M. smegmatis* with MIC of 1000 µg/mL (Mariita et al. 2010). Fabry et al. (1998) and Eleazar et al. (2020) indicated that the methanolic stem bark extract, methanolic and ethyl acetate leaf fractions of this species displayed antimicrobial effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococci*, *Salmonella* and *Klebsiella* species with MIC of 0.25–8.0 mg/ml. Taken together, all the extracts tested elicited inferior antimycobacterial activity when compared to rifampicin ($P < 0.01$). This may be the case because crude samples have matrix interferences which reduces the overall concentration of bioactive compounds at the target to produce the desired bioactivity.

Table 1 Minimum inhibitory and median cytotoxic concentrations of *E. abyssinica* stem bark extracts

Extract/drug	Minimum inhibitory concentration (µg/mL)		Median cytotoxic concentration (µg/mL)
	M. smegmatis	*Mtb*	Vero E6 cells
Hexane	2500.0 ± 0.0 [a]	?	841.29 ± 37.76 [a]
Chloroform	2500.0 ± 0.0 [a]	–	> 1000 [a]
Acetone	1250.0 ± 0.0 [a]	937.0 ± 442.0 [a]	677.65 ± 68.93 [a]
Methanol	1250.0 ± 0.0 [a]	468.0 ± 22.0 [a]	> 1000 [a]
Rifampicin	15.0 ± 0.0	4.0 ± 0.0	520.02 ± 40.11

[a] significant at $P < 0.01$ for extract vs. rifampicin; – means not active.

All the four extracts of *E. abyssinica* stem bark had relatively low toxicity against Vero E6 cells ($CC_{50} > 500$ µg/mL) when compared with rifampicin ($P < 0.01$) according to the classification of Cytotoxicity is categorized based on CC_{50} as follows by the National Cancer Institute i.e. IC_{50} of 201–500 µg/mL = weakly cytotoxic (Erhirhie et al. 2018). This suggests that *E. abyssinica* stem bark is devoid of toxic phytochemicals that may be harmful when the used in herbal medicine, corroborating our previous in vivo observations (Obakiro et al. 2021). The median lethal dose of *E. abyssinica* leaf fractions were recently reported to be 2154 mg/kg, implying that it is slightly toxic (Eleazar et al. 2020). In contrast, several compounds isolated from *E. abyssinica* leaves were reported to be moderately cytotoxic against Vero cells with CC_{50} ranging between 22.42 and 80.55 µg/mL (Dzoyem et al. 2017).

Phytochemical profile of*E. abyssinica*stem bark extracts

Secondary metabolites identified

The most abundant secondary metabolites detected were triterpenes, coumarins, tannins, flavonoids and alkaloids. Acetonic extract had the highest abundance whereas hexanic extract had the least (Table 2). These abundances could be related to the differences in the solvent polarities (Koparde et al. 2017; Omara et al. 2021) and maybe be implicated in the observed antimycobacterial activities. Classical phytochemical analysis of methanolic and ethyl acetate leaf fractions of *E. abyssinica* showed the presence of alkaloids, flavonoids and steroids (Eleazar et al. 2020). Structural elucidation of compounds in *E. abyssinica* stem bark and leaf extracts reported flavonoids, terpenoids, the diterpene klovaol and kolavic acid derivatives as the major phytochemicals (Debella et al. 2000; Dzoyem et al. 2017; Freiburghaus et al. 1998; Nyasse et al. 2004; Sobeh et al.

Table 2 Secondary metabolites identified in stem bark extracts of *E. abyssinica**

Phytochemical class	Acetonic extract	Methanolic extract	Chloroform extract	Hexane extract
Volatile oils	+	–	++	+++
Triterpenes	++	+	+++	++
Flavonoids	+++	+	++	++
Alkaloids	++	+	++	+
Coumarins	++	++	++	–
Steroid glycosides	++	++	–	–
Anthocyanins	++	–	–	–
Anthracenocides	+	+	–	–
Reducing sugars	++	++	–	–
Saponins	++	+	–	–
Tannins	+++	++	–	–

*$^{+++}$ represent *abundant*, $^{++}$ represent *moderate*, $^{+}$ represent *traces*, and $^{-}$ represent *absent* based on colour or foam intensities.

2020). These reports are in agreement with our observation in this study.

FT-IR and GC-MS results of bioactive extracts

In the FTIR spectrum of acetonic extract, the peaks at 1021.12 cm^{-1} and 1203.36 cm^{-1} were assigned to C-O bonds whereas the that at 1337.39 cm^{-1} corresponded to a C-O-H bond. Other peaks were 1444.56 cm^{-1} (for C=C), 1604.45 cm^{-1} (for C=C or C=O) and 3205.11 cm^{-1} for O-H (Fig. 1a). For the methanolic extract, the wavenumbers 1143.58 and 1024.02 cm^{-1} could be assigned to C-O bonds. A typical C-O-H bond could be assigned at 1316.17 cm^{-1}. Similarly, 1445.38 cm^{-1} corresponded with C=C while those observed at 1694.15 cm^{-1} and 3198.15 cm^{-1} were assigned to C=C or C=O and O-H, respectively (Fig. 1b).

For GC-MS analysis, derivation was performed in this study. Phytochemical derivatization using appropriate agents

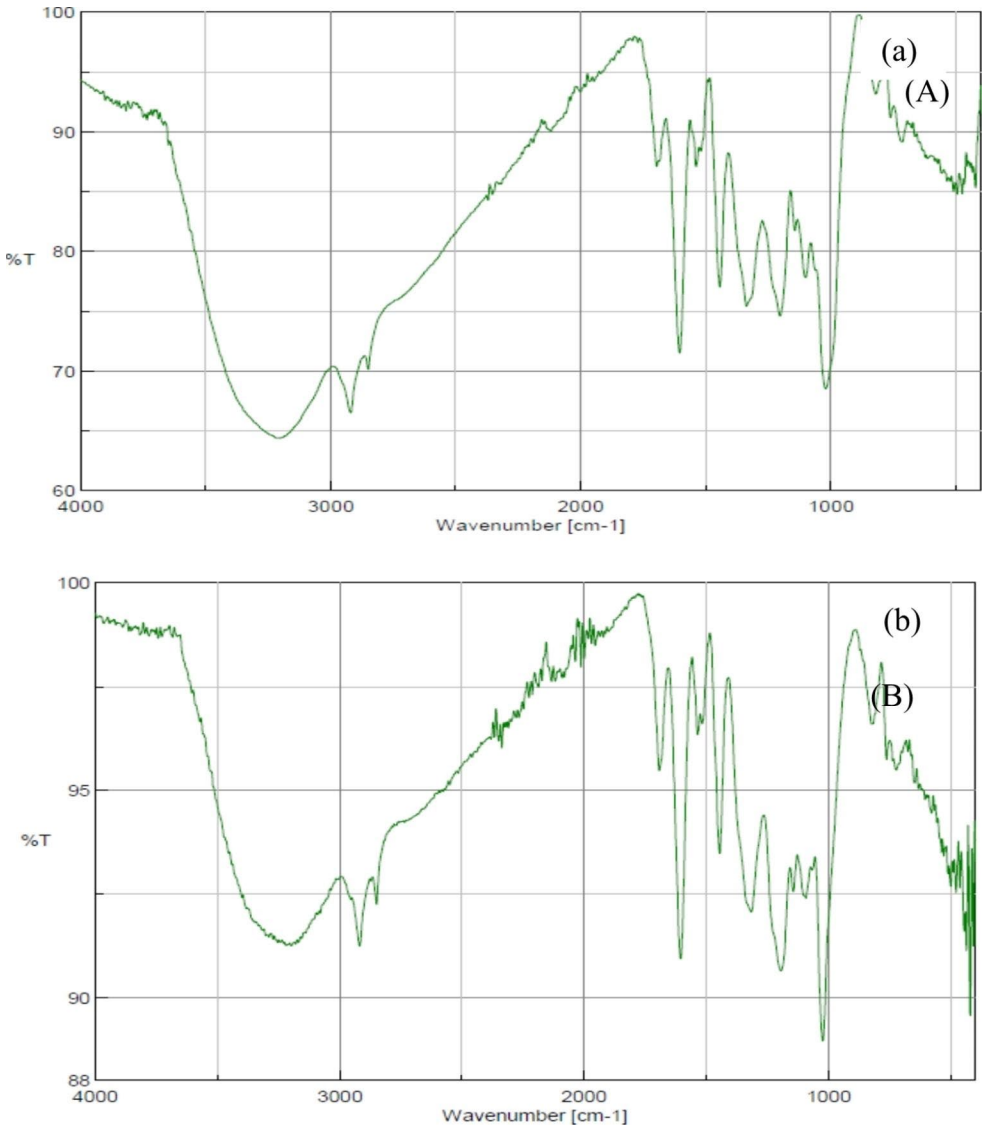

Fig. 1 FTIR spectra of (a) acetone, and (b) methanolic extracts of *Entada abyssinica*

is an emerging technique for increasing GC-MS detection. To the best of our knowledge, this is the first report on GC-MS analysis of stem bark extracts of *E. abyssinica.* Chromatograms of the acetonic and methanolic extracts of *Entada abyssinica* revealed a total of 14 and 13 peaks, respectively which corresponded with authentic compounds in NIST library (**Supplementary Table S1;** Fig. 2). Phenyl 4-[(trimethylsilyl)amino]-2-[(trimethylsilyl)oxy]benzoate (**1**); 1 H-indene, 2,3-dihydro-1,4,7-trimethyl- (**2**); 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (**3**); 9-octadecenamide, (Z)- (**4**); 2-benzyl-4-oxo-4-phenyl-butanoic acid (**5**); indan,1-(2-methylpropenyl)-2-thiocyanato- (**6**), hexadecanoic acid, methyl ester (**7**), oleic acid (**8**), Carnegine (**9**) (Fig. 2a) were the most abundant in acetonic extract. In the methanolic extract, 5-hydroxymethylfurfural (**10**); 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (**11**); hexadecanoic acid, 14-methyl-, methyl ester (**12**); 9,12-octadecadienoic acid (Z,Z)-, methyl ester (**13**) and methyl stearate (**14**) were the dominant compounds. These compounds are majorly esters, which is in agreement with previous studies which indicated that the stem bark of *E. abbysinica* contained 1',26'-bis-[(S)-2,3-dihydroxypropyl] hexacosanedioate (a monoglyceride), entadanin (a peltogynoid), linoleic acid, β-sitosterol, pentacosanoic acid, stigmasterol, methyl gallate, clerodane-type diterpenes (8 S-kolavic acid 15-methylester; 13,14,15,16-tetranor-3-clerodene-12,18-dioic acid, and 14,15,16-trinor-3-clerodene-13,18-dioic acid), flavonoids (flavanones) and phytosterol glycosides (Debella et al. 2000; Melong et al. 2014; Nyasse et al. 2000; Teke et al. 2011).

All the compounds that were identified by GC-MS correlated with the functional groups obtained by FTIR spectroscopy. Out of the 14 compounds identified in the extracts, three compounds: hexadecanoic acid, methyl ester (7), oleic acid (8) and 5-hydroxymethylfurfural (10) have previously been reported to elicit antimicrobial activity against an array of bacterial and fungal pathogens (Leyton et al. 2011; Shaaban et al. 2021). Oleic acid (8) particularly had antimycobacterial activity against susceptible and multidrug-resistant strains of Mtb with MIC of 100 µg/mL (Esquivel-Ferriño et al. 2012). In another study, oleic acid (8) showed 100% inhibition of the growth of Mtb with MIC of 25 µg/mL (Saravanakumar et al. 2008). These bioactivities suggest that E. abyssinica stem bark has the potential to provide novel antimycobacterial agents.

Conclusion

In this study, acetonic, chloroform, methanolic and hexanic extracts of *E. abyssinica* stem bark were screened for their antimycobacterial, cytotoxicity and bioactive

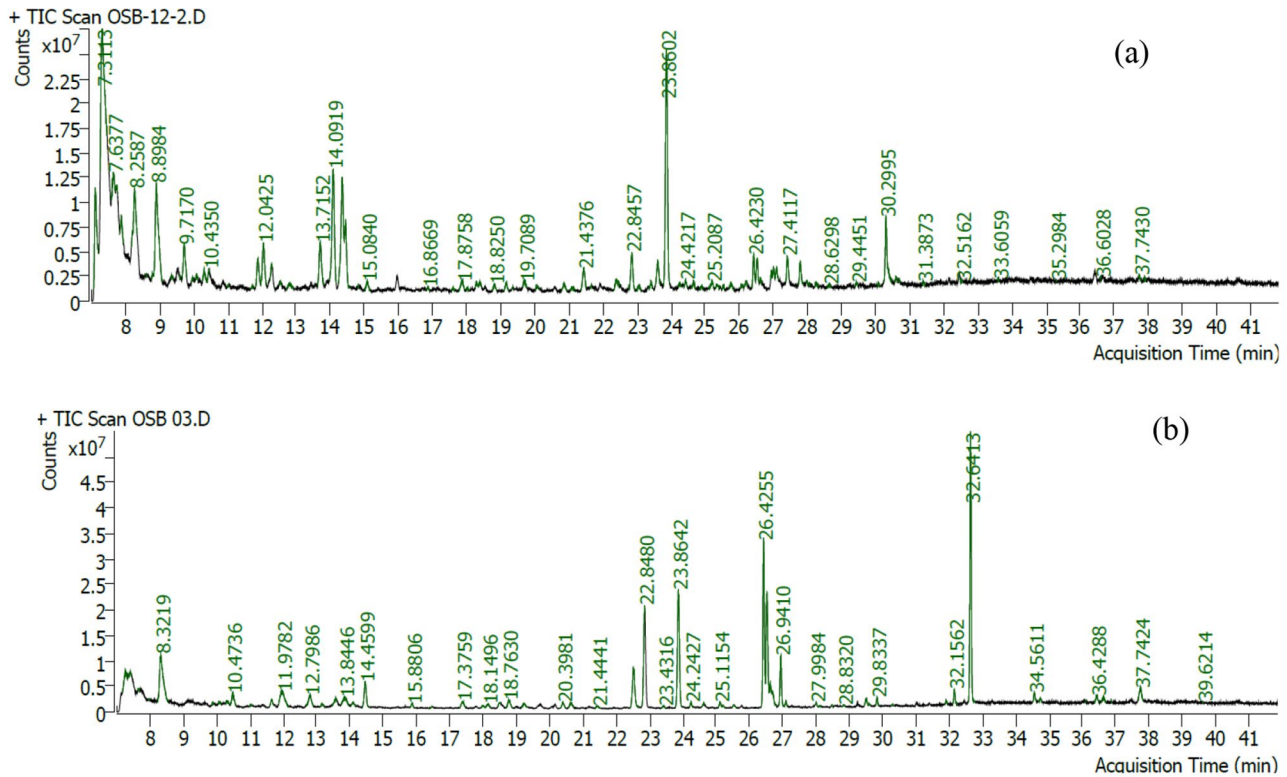

Fig. 2 Gas chromatogram of compounds identified in (**a**) acetonic, and (**b**) methanolic extracts of *E. abyssinica* stem bark

phytochemicals. Acetonic and methanolic extracts were the most bioactive (MIC range: 125 to 468 µg/mL) while the CC_{50} of all the extracts were greater than 500 µg/mL. GC/MS analysis revealed 14 compounds in the acetonic and methanolic extracts. Of these, a known antimycobacterial compound (oleic acid) was identified. These results show that acetonic and methanolic extracts of *E. abyssinica* stem bark possess promising antimycobacterial activity against *M. smegmatis* and *Mtb*, lending credence to the use of this species in the managing symptoms of TB in East Africa. Therefore, further studies investigating the antimycobacterial potential and safety of pure compounds isolated from this species should be initiated.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42535-023-00732-z.

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Declarations

Ethical approval Approval for this study was granted by the Scientific and Ethics Research Unit of Kenya Medical Research Institute, Kenya (KEMR/SERU/CTMDR/CSCP085/4067).

Conflict of interest The authors declare that no conflict of interest exists.

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