

# Anti-plasmodial, anti-trypanosomal and cytotoxic effects of *Anthonotha macrophylla*, *Annickia polycarpa*, *Tieghemella heckelii* and *Antrocaryon micraster* extracts

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## Research Article

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# Abstract

Malaria and trypanosomiasis are protozoan diseases which pose a devastating challenge to human health and productivity especially, in Africa where their respective vectors (Female *Anopheles* mosquito and tsetse fly) abound. Various medicinal plants are used to treat these parasitic diseases. However, the scientific basis of their use and toxicological profiles have not been assessed. We have therefore, evaluated the anti-plasmodial, anti-trypanosomal and cytotoxicity activities of four African medicinal plant extracts namely, *Anthonotha macrophylla* leaf (AML), *Annickia polycarpa* leaf (APLE), *Tieghemella heckelii* stem bark (THBE) and *Antrocaryon micraster* stem bark (AMSBE) extracts in vitro against *P. falciparum* (W2mef laboratory strain), *T. brucei* (GUTat 3.1 strain) and mammalian RAW 264.7 macrophage cell line respectively. The most active anti-plasmodial extract was AML ( $IC_{50} = 5.0 \pm 0.08$   $\mu\text{g/mL}$  with SI of 21.9). THBE also, produced the most effective ant-trypanosomal activity ( $IC_{50} = 11.0 \pm 0.09$   $\mu\text{g/mL}$  and SI of 10.2) among the extracts. In addition, none of the extracts produced toxic effect in RAW 264.7 macrophage cell line except APLE which was moderately cytotoxic and also produced the least SI in both anti-trypanosomal and anti-plasmodial assays. These results suggest that AML and THBE could offer safe and alternative therapy for malarial and trypanosomiasis. This is the first study to report the anti-trypanosomal and in vitro anti-plasmodial activities of these four plants. The cytotoxicity of the plant parts used are also being reported for the first time except for *T. heckelii* stem bark.

## 1. Introduction

Malaria is caused by several species of protozoan parasites from the genus *Plasmodium*. In sub-Saharan Africa, the most pathogenic species is the *Plasmodium falciparum* which causes more than 90% of all malaria cases (WHO, 2019). The World Malaria Report reported an estimated 241 million malaria infections and 627 000 malaria deaths globally in 2020, which denotes about 14 million increased in cases and 69 000 additional deaths, in 2020 as against 2019 (WHO, 2021). Even though the development of resistance, by the *Plasmodium* parasite, to artemisinin and its combination therapies (ACTs) in Sub-Saharan Africa is slower compared to places like Asia and South America, the burden of malaria in the former region remains high (Bushman et al., 2016; WHO, 2019; Edwin et al., 2019).

African trypanosomiasis, a tsetse-transmitted disease of humans and livestock caused by protozoan parasites of the genus *Trypanosoma*, is of serious health and economic concerns in various sub-Saharan African countries (Simarro et al., 2012; Morrison et al., 2016). In the absence of vaccines, chemotherapy remains the only practical means to control African trypanosomes (Stervedin, 2010). However, development of resistance to present drugs, side effects and difficulty in regimen application pose serious challenges to chemotherapy (Scott et al., 1996; Matovu et al., 2001; Barrett et al., 2011; Franco et al., 2012).

Macrophages are mononuclear phagocytes that form critical components of the mammalian immune system. RAW cells are macrophages originally established from ascitic tumor induced in a male BALB/c mouse by intraperitoneal injection of Abelson leukemia virus (Raschke et al., 1978). RAW 264.7 cell lines

are useful in the evaluation of the bioactivity of natural products as well as envisage their probable in vivo outcomes (Merly and Smith, 2017) and toxicity. Thus, they provide useful advantages in drug discovery research.

Folkloric medicinal plants have proven to be the most versatile source of crude and purified drugs for mankind. Furthermore, it has been documented that approximately 60% of pharmaceutical drugs being used currently were directly or indirectly derived from plants and other natural origins (Newman, 2008). Due to the challenges associated with current anti-malaria and anti-trypanosomal drugs, the need to find alternate and new medications to combat these diseases, especially, from folkloric medicinal plants has become a focus of global scientific research.

Four anti-malaria folkloric African medicinal plants have been selected for this study. Malaria and trypanosomiasis are both protozoan diseases. Hence, the extracts were tested against both parasites. The selected medicinal plants are discussed below.

*Anthonotha macrophylla* (P. Beauv), Caesalpiniaceae is an evergreen tree (Zirihi et al., 2005) whose leaf is a remedy for diarrhoea, dysentery, skin infections headache and obnoxious stings (Keay, 1959; Burkill, 1985; Mshana et al., 2000). The stem bark is used to treat venereal diseases and malaria. So far, aphrodisiac, safety, anti-inflammatory, analgesic and antioxidant activities of the leaf in addition to anti-plasmodial activity of the stem bark have been reported (Yakubu and Abdulquadri, 2016; Essien and Sam, 2018; Zirihi et al., 2005; Kumatia et al., 2021a). *Antrocaryon micraster* (A. Chev. and Guillaumin), Anacardiaceae, is a timber species, whose stem bark is used to treat malaria, impotence, bodily pains and arthritis (Vigbedor et al., 2008; Ayarkwa, 2011; Ayarkwa, 2012). Anti-inflammatory, anti-oxidant and in vivo anti-malaria activities were reported for the plant (Essel et al., 2017; Kumatia et al., 2021b).

*Annickia polycarpa* ((DC.) Setten and Maas ex I.M. Turner.) a member of the Annonaceae family, is employed to treat malaria, fever, stomach ulcer, pyrexia, bacterial infections, injuries, eye infections and wounds (Irvine, 1961; Ajali, 2000; Atindehou et al., 2004; Govindasamy *et al.*, 2007). Anti-malaria, anti-trypanosome, analgesic, anti-inflammatory and anti-bacterial activities have been reported of the plant (Ajali, 2000; Atindehou et al., 2004; Bolou et al., 2011; Anosa et al., 2014; Kumatia et al., 2016; Kumatia et al, 2021c). The stem bark of *Tieghemella heckelii*, (A. Chev.) Pierre ex. Dubard, a timber species of the Sapotaceae family, is used to treat toothache, blennorrhoea and malaria whereas the seed is employed against hernia (Burkill, 2000; CSIR-FORIG, 2019). The stem bark of *T. heckelii* have been reported to possess cytotoxic, anti-bacterial, analgesic and anti-inflammatory activities (Kipre et al., 2017; Kumatia and Appiah-Opong, 2021).

Although, the in vivo anti-malaria activity of *A. micraster* stem bark, *A. polycarpa* stem bark and leaf in addition to the in vitro anti-malaria activity of *A. macrophylla* stem bark have been reported, the anti-trypanosomal activity of the four plants and/or plant parts is not known. Additionally, the in vitro anti-plasmodial activity and selectivity index (SI) of the selected plant parts have also not been reported. This study therefore, sought to evaluate the in vitro anti-plasmodial, anti- trypanosomal and cytotoxic activities

of *A. macrophylla* leaf (AML), *A. micraster* stem bark (AMSBE), *A. polycarpa* leaf (APLE), *T. heckelii* stem bark (THBE).

## 2. Materials And Methods

### 2.1. Chemicals and Reagents

HMI9, FBS and DMEM were purchased from Thermo Fisher scientific, UK. Other chemicals and reagents such as penicillin-streptomycin-L-glutamine (PSG), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), chloroquine, diminazene aceturate, alamar dye, SYBR Green I, dimethyl sulphoxide (DMSO), sodium citrate, adenine, sodium bicarbonate ( $\text{NaHCO}_3$ ), sodium chloride (NaCl), sodium phosphate dibasic ( $\text{Na}_2\text{HPO}_4$ ), potassium chloride (KCl), sodium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ), sodium hydroxide (NaOH), and sodium bicarbonate ( $\text{NaHCO}_3$ ) were also purchased from Sigma-Aldrich, UK.

### 2.2. Identification, Collection, Processing and Extraction of Plant Materials

The plants were identified and collected by Mr. Jonathan Dabo, a botanist at the Forestry Research Institute of Ghana (FORIG) Kumasi. *A. macrophylla* leaf, *A. polycarpa* leaf and *T. heckelii* stem bark were collected from Bobiri Forest Reserve in the Ashanti Region of Ghana. *A. micraster* stem bark was collected from Asubima Forest Reserve in the Ahafo Region of Ghana. The plant materials were sun dried for eight days and pulverized into coarse powder. *A. polycarpa* leaf, *T. heckelii* and *A. micraster* stem barks were assigned voucher specimen numbers (FORIG 0012, FORIG 0013 and FORIG 0014 respectively) and deposited at the herbarium of FORIG. *A. macrophylla* leaf was given a voucher specimen number of CPMR 4937 at the herbarium of Centre for Plant Medicine Research, Mampong-Akwapim, Ghana. The plant materials were sun dried for 8 days and pulverized into course powdered. Extraction was performed using the protocol of Kumatia et al., (2021a). Briefly, the powdered stem bark of *A. micraster* (200 g) was extracted with 70% ethanol (2 L x 2) at room temperature for four days each filtered and combined. The ethanol in the extract was removed by concentrating the filtrate at low pressure in a rotary evaporator (Eyeler N1110, Tokyo-Japan). The aqueous part was lyophilized to obtain a powder coded AMSBE. Similar procedure was applied to the other plant materials to obtain solid extracts coded APLE, THBE and AML for *A. polycarpa* leaf, *T. heckelii* stem barks and *A. macrophylla* leaf respectively. The extracts were stored in air-proof containers under 4 °C until required.

### 2.3. Phytochemical screening of the extracts

An aliquot of each extract (50 mL) was analyzed for the presence or absence of phytochemical compounds such as flavonoids, alkaloids, triterpenes, phenolic compounds, saponins, polyuronides, anthracinosides, reducing sugars, phytosterols and cyanogenic glycosides according using the methods described by Fong et al., (1977).

### 2.4. Culture of *Plasmodium falciparum*

W2mef laboratory strain of *P. falciparum* was cultured with human group O<sup>+</sup> erythrocytes using standard methods (Trager and Jensen, 1976) with slight modifications. *P. falciparum* parasites were then cultured to > 5% parasitemia of ring stage parasites. Using 5% sorbitol treatment, a synchronized culture of ring-stage parasites (Lambros and Vanderberg, 1979) was obtained and diluted to 1% parasitemia and 2% haematocrit for the growth inhibition assays.

## 2.5. Cell viability analysis of *Plasmodium falciparum*

Extracts were tested against *P. falciparum* activity in the SYBR Green I fluorescence assay. Extracts at concentrations of 10 mg/mL were diluted with culture media to a starting working concentration of 100 µg/mL. Serial dilutions (1:2) were made to yield 7 final concentrations each of the extract (100 – 0.78 µg/mL). An aliquot of 10 µL of each concentration was dispensed into test wells of 96-well plate in triplicates with each test well already containing 90 µL of 2% hematocrit and 1% parasitemia. Wells containing RBCs at 2% hematocrit and 1% parasitemia were used. Final volume per well was 100 µL. Plates were incubated for 48 h and an aliquot of 100 µL of 4x buffered SYBR Green I (0.25 µL of 10,000X SYBR Green I/mL of 1X phosphate buffer saline) was added to each well after the incubation period and incubated again in the dark for 30 min at 37 °C. The presence and amount of infected red blood cells (RBCs) was detected using BD FACS LSRFortessa™ X-20 flow cytometer and analyzed with BD FACSDiva Software (v8.0.1). A total of 5,000 RBCs were counted to determine the number of infected RBCs (SYBR Green I positive cells) present. Chloroquine (CHL) was used as the reference anti-plasmodial drug.

## 2.6. Culture of *Trypanosoma brucei*

Blood stream forms of the subspecies *T. brucei* (GUTat 3.1 strains) were cultured *in vitro* to the logarithm phase using Hirumi's Modified Iscove's Medium (HMI9, Thermo Fisher Scientific) with 10% foetal bovine serum (Thermo Fisher Scientific) at 5% CO<sub>2</sub> and 37 °C.

## 2.6. Cell viability analysis of *Trypanosoma brucei*

Cells were seeded at a density of 3.0x10<sup>5</sup> cells/ml on 96-well plates in a two-fold dilution of extracts. Extracts were incubated in a two-fold dilution with the cells for another 24 h. Alamar blue dye (resazurin, 10% v/v) was then added to all wells and incubated for another 24 h. Spectrophotometric absorbance was recorded at a wavelength of 570 nm. Diminazene aceturate (DA) was used as a positive anti-trypanosomal control drug.

## 2.7. Culture of mouse macrophages (RAW 264.7)

Mouse macrophages (RAW 264.7 cell lines) were cultivated *in vitro* to the logarithm phase using Dulbecco's Modified Eagle Media (DMEM, Thermo Fisher Scientific) with 10% foetal bovine serum at 5% CO<sub>2</sub> and 37°C.

## 2.8. Cytotoxicity analysis of mouse macrophages (RAW 264.7)

Cell lines were plated at a density of  $3.0 \times 10^5$  cells/mL for 48 h to allow for sufficient adherence to plates. Extracts were incubated in a two-fold dilution with the cells for another 24 h. Alamar blue dye (resazurin, 10% v/v) was then added to all wells and incubated for another 24 h to allow for a complete color. Spectrophotometric absorbance was recorded at a wavelength of 570 nm.

## 2.9. Selectivity Index (SI)

SI was calculated for each extract in each test to determine their effectiveness and toxicity in their use as anti-plasmodial or anti-trypanosomal agent using the formular below.

$$SI = \frac{IC_{50} \text{ obtained for the extract in the RAW macrophage cell}}{IC_{50} \text{ obtained for the extract against the protozoan parasite}}$$

## 2.10. Statistical Analysis

Algorithms obtained from flow cytometry (FACS) were analyzed using regression equations of best fit of plotted growth inhibition versus concentration curves. Graph Pad Prism for Windows version 7.0 (Graph Pad Software, San Diego, CA, USA) was used for all statistical analyzes. Cell viability of parasites was estimated from the fluorescence of SYBR Green I or absorbance of resazurin and represented as a percentage of treated to untreated cells. IC<sub>50</sub> values were calculated from a non-linear regression model. Data were presented as mean ± S.E.M

## 3. Results And Discussion

### *Yield and nature of crude extracts*

The weight, yield and nature of the extracts obtained are described below in Table 1. The result shows that the stem barks produced higher yields of extracts than the leaves.

Table 1  
Yield and nature of solid extracts produced

| Extract | Weight extracted (g) | Weight of extract (g) | Yield (%w/w) | Color of extract |
|---------|----------------------|-----------------------|--------------|------------------|
| AMSBE   | 200                  | 41.29                 | 20.65        | Dark brown       |
| AML     | 200                  | 9.43                  | 4.72         | Green            |
| THBE    | 200                  | 43.76                 | 21.88        | Reddish brown    |
| APLE    | 200                  | 31.56                 | 15.78        | Green            |

The stem bark extracts yielded more extracts than the leaves under the same conditions. This was due to the fat that most trees have very tick and malleable stem barks. THBE produced the highest yield of extract (21.88% w/w). This was followed closely with AMSBE. AML produced the least yield of extract

(4.72% w/w). This indicates that less plant material is needed to produce enough extract for the plant parts that gave high yield and vice versa.

### Classes of phytochemical constituents in the extracts

The results of the phytochemical analysis of the extracts are tabulated below in Table 2. The results show that all the extracts contained saponins, phenolics and reducing sugars. Phytosterols were found in all the extracts except AMSBE. AML and THBE also contained flavonoids in addition to the other four constituents. Alkaloids were also present in APLE, which contained the greater number of phytochemical constituents among the extracts.

Table 2  
Phytochemical screening results of the extracts

| Phytochemical constituent | AMSBE   | AML     | THBE    | APLE    |
|---------------------------|---------|---------|---------|---------|
| Saponins                  | Present | Present | Present | Present |
| Phenolic compounds        | Present | Present | Present | Present |
| Free reducing sugars      | Present | Present | Present | Present |
| Flavonoids                | Absent  | Present | Present | Absent  |
| Phytosterols              | Absent  | Present | Present | Present |
| Alkaloids                 | Absent  | Absent  | Absent  | Present |
| Antracenosides            | Absent  | Absent  | Absent  | Absent  |
| Polyuronoids              | Absent  | Absent  | Absent  | Absent  |
| Triterpenes               | Absent  | Absent  | Absent  | Absent  |
| Cyanogenic glycosides     | Absent  | Absent  | Absent  | Absent  |

The yield, nature and phytoconstituents obtained for the extracts were similar to those reported elsewhere (Kumatia et al.,2021a; Kumatia et al, 2021b; Kumatia et al, 2021c) because it was the same bath of plant material used in both studies.

### Anti-plasmodial, anti-trypanosomal and cytotoxic activities

The results of the in vitro anti-plasmodial, anti-trypanosomal and cytotoxic activities of the extracts are shown in Table 3 below.

Table 3  
IC<sub>50</sub> of the extracts against *P. falciparum*, *T. brucei* and  
RAW 264.7 cell line inhibitions

|          | <i>P. falciparum</i>     | <i>T. brucei</i> | Cytotoxicity |
|----------|--------------------------|------------------|--------------|
| Extracts | IC <sub>50</sub> (µg/mL) |                  |              |
| AML      | 5.0 ± 0.08               | 13.6 ± 0.21      | 109.4 ± 0.12 |
| APLE     | 110.1 ± 0.12             | 53.0 ± 0.05      | 76.5 ± 0.22  |
| THBE     | 28.8 ± 0.16              | 11.0 ± 0.09      | 112.0 ± 0.32 |
| AMSBE    | 24.5 ± 0.21              | 19.9 ± 0.15      | 98.5 ± 0.24  |
| DA       | –                        | 1.1 ± 1.13       | 74.1 ± 0.31  |
| CHL      | 0.146 ± 0.05             | –                | 6.88 ± 0.32  |

The extracts produced anti-plasmodial activity with IC<sub>50s</sub> of 5.0 ± 0.08–110.1 ± 0.12 µg/mL and that of CHL was 0.146 ± 0.05 µg/mL. AML was the most active extract. The extracts can be arranged in increasing order of anti-plasmodial activity as follows: APLE < AMSBE < THBE < AML. According to Zirihi et al., (2005), the in vitro anti-plasmodial activity of plant extracts are classified as good, weak or in active using their IC<sub>50</sub> values. IC<sub>50</sub> value of greater than 50 µg/mL, 15–50 µg/mL or less than 15 µg/mL indicates inactive, weak or good anti-plasmodial activity respectively. Thus, AML (IC<sub>50</sub> = 5.0 ± 0.08 µg/mL) produced good anti-plasmodial activity. THBE and AMSBE (IC<sub>50</sub> = 28.8 ± 0.16 and 24.5 ± 0.21 µg/mL respectively) (Table 3) produced weak in vitro anti-plasmodial activity. Whereas, APLE (IC<sub>50</sub> = 110.1 ± 0.12 µg/mL) was inactive. AMSBE and APLE were shown to demonstrate significant anti-malaria activity in mice in vivo (Kumatia et al., 2021a; Kumatia et al., 2021b). However, these extracts were inactive against *P. falciparum* in vitro. This suggests that the extracts act as prodrugs in vivo where their metabolites might be responsible for their observed anti-malaria action.

Furthermore, the extracts produced anti-trypanosomal activity with IC<sub>50</sub> values of 13.6 ± 0.21–53.0 ± 0.05 µg/mL and that of the reference drug (DA) was 1.1 ± 1.13 µg/mL (Table 3). The extracts can be arranged in increasing order of anti-trypanosomal activity as follows: APLE < AMSBE < AML < THBE. Anti-trypanosomal activity of medicinal plant extract is classified as good, weak or inactive when IC<sub>50</sub> values of less than 8.0 µg/mL, ranging from 8.1–25.0 µg/mL or greater than 25 µg/mL is respectively obtained (Atindehou et al., 2004). Therefore, three out of the four extracts in this study (AMSBE, AML and THBE) produced weak anti-trypanosomal activity (IC<sub>50</sub> = 11.0 ± 0.09–19.9 ± 0.15 µg/mL). Whereas APLE (IC<sub>50</sub> = 53.0 ± 0.05 µg/mL) was inactive.

The most important parameter in cytotoxicity test is the IC<sub>50</sub> value which is defined as the concentration of a test substance which can inhibit the viability of 50% of a cell under specific test conditions. IC<sub>50</sub> values are used to measure the toxicity or safety of chemical agents in cytotoxicity studies using cell

lines. The smaller the IC<sub>50</sub> value of a substance, the more toxic is the substance. Conversely, the higher the IC<sub>50</sub> value of a test substance, the safer/less toxic is that substance. According to the WHO a substance is classified as non-toxic, relatively toxic or toxic when it recorded an IC<sub>50</sub> of greater than 90.0, between 89.0–2.0 or less than 2.0 µg/mL respectively on L-6 rat skeletal myoblast/mammalian cells limes (WHO, 2017). In this study, AMSBE, AML and THBE gave IC<sub>50</sub> values of 98.5 ± 0.24–112.0 ± 0.32 µg/mL. Hence, they are nontoxic (Table 3). APLE and the reference drug DA gave IC<sub>50</sub> values of 76.5 ± 0.22 and 74.1 ± 0.31 µg/mL respectively (Table 3). This makes them relatively toxic.

### Selectivity index (SI) of the extracts

The activity and toxicity profiles in the parasites and macrophages resulted in the list of selectivity profiles shown in Table 4.

| Table 4<br>Selectivity Index (SI) of the extracts |               |           |
|---------------------------------------------------|---------------|-----------|
| SI                                                |               |           |
| Extracts                                          | P. falciparum | T. brucei |
| AML                                               | 21.9          | 8.0       |
| APLE                                              | 0.7           | 1.4       |
| THBE                                              | 3.9           | 10.2      |
| AMSBE                                             | 4.0           | 4.9       |
| DA                                                | N/A           | 67.4      |
| CHL                                               | 47.12         | N/A       |
| NA = not applicable.                              |               |           |

SI is the ratio of the IC<sub>50</sub> in mice macrophage RAW 264.7 to the IC<sub>50</sub> in the respective parasite (*P. falciparum* or *T. brucei*). Higher SI of a substance suggests the likelihood that the substance could produce innocuous treatment. And the smaller the SI of a substance, the more likely it is for that substance to produce ineffective and harmful treatment. The results obtained from this study therefore, indicates that AML (anti-plasmodial SI = 21.9) and THBE (anti-trypanosomal SI = 21.9) which recorded the highest SI against *P. falciparum* and *T. brucei* respectively among the extracts as the most promising in vitro anti-plasmodial and anti-trypanosomal agents respectively. APLE emerged as the least promising anti-trypanosomal and antiplasmodial agent since it has the least SI of 1.4 and 0.7 respectively in both tests. It was suggested that a substance with an SI value < 2 might produce wide-ranging toxicity (Koch et al., 2005). Therefore, the extracts are safe in vitro except APLE which could be classified as generally toxic in vitro.

The phytochemical analysis results showed that only AML and THBE have flavonoids present in them whereas three to four other constituents were common to all the extracts. AML and THBE are the most active extracts in this study. This indicates that flavonoids may be responsible for the anti-plasmodial and anti-trypanosomal activity demonstrated by the two extracts in this study. Flavonoids such as apigenin, genkwanin, scutellarein and diosmetin were reported to produce anti-trypanosomal activity against *T. brucei* in vitro with IC<sub>50</sub> of 5.1, 8.0, 4.6 and 6.1 µg/mL, respectively (Tasdemir et al., 2006).

## Conclusion

The results from this study indicates that AML possessed good anti-plasmodial activity, weak anti-trypanosomal activity and high SI among the extracts in the anti-plasmodial test. THBE also, produced good ant-trypanosomal activity with the highest SI of 10.2 among the extracts. None of the extracts showed cytotoxic effect in the RAW 264.7 macrophage cell line expect APLE which was moderately cytotoxic and also produced the least SI in both anti-trypanosomal and anti-plasmodial tests. These results suggest that AML and THBE could offer safe and alternative therapy for malaria and trypanosomiasis. This is the first study to report the anti-trypanosomal and in vitro anti-plasmodial activities of these four plants. The activity of AML and THBE may be due to their chemical constituents such are flavonoids which were absent in the other extracts. The AML and THBE could also to fractionated to isolate their constituents for pre-clinical anti-plasmodial and anti-trypanosomal studies.

## Declarations

### Funding information

This study was supported by the authors.

### Data Availability

The data upon which the conclusions in this study are drawn can be obtained from the corresponding author upon reasonable request.

### Conflict of Interest

The authors declare that they have no competing interests.

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