

(RESEARCH ARTICLE)



Evaluation of the antioxidant and antibacterial activity *in vitro* of the trunk bark of *Canarium schweinfurthii* Engl (Burseraceae)

Valère Igor Deumi Monthe^{1,2}, Patrick Yamen Mbopi^{1,*}, Cyrielle Signe Mawout², Jean Jacques Tchouani¹, Pierre Réne Fotsing Kwetche² and Jeanne Yonkeu Ngogang³

¹ Laboratory of Pharmacognosy, Higher Institute of Health Sciences, Université des Montagnes, B.P. 208 Bangangté; Cameroon.

² Laboratory of Microbiology, Higher Institute of Health Sciences, Université des Montagnes, B.P. 208 Bangangté; Cameroon.

³ Department of Physiological Sciences and Biochemistry, Faculty of Medicine and Biomedical Sciences, University of Yaounde I, P.B. 1364 Yaounde; Cameroon.

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Abstract

The high incidence of infectious diseases and the emergence of resistance to modern drugs are current public health concerns. This situation leads to the search for alternatives via medicinal plants. To investigate the antibacterial and antioxidant activities of hydroalcoholic extract of *Canarium schweinfurthii* trunk bark commonly used in traditional medicine for the treatment of various pathologies. The primary phytochemical study was performed on hydro alcoholic extract of the trunk bark dried in the shade. The antioxidant activity of the extract was evaluated using ferric reducing power and the free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the antibacterial test and MIC was determined by using dilution methods against twenty-two strains of bacteria. The phytochemical screening has shown the presence of phenolic compounds, flavonoids, tannins, terpenoids, alkaloids and steroids. The antioxidant activity demonstrated that the extract at the concentration of 500 µg/mL scavenges DPPH and the ferric ion by 65.07%, and 90.36% of the radicals respectively. The antibacterial activity demonstrated that the extract inhibited the strains used independently of the gram with MICs varying between 1.95 and 31.25 mg/mL. The extract also showed an absolute bactericidal potential on multiresistant strains. The antibacterial potential could be due to the presence of phenolic compounds. The activities of hydro-ethanolic extracts of *C. schweinfurthii* suggest that the latter would be a potential raw material for the production of improved traditional medicines

Keywords: *Canarium schweinfurthii*; Hydroethanolic extract; Antioxidant activity; Antibacterial activity

1. Introduction

It is now well known that radicals play a fundamental role in several diseases [1] The biochemical damage they cause to cells and tissues has been implicated in several pathological disorders in humans and animals, including cancer, hypertension, diabetes, neurodegenerative disorders, inflammatory and infectious diseases [2,3,4]. The resistance of bacteria to the available synthetic and semi-synthetic antibacterial agents is growing rapidly [5]. It has been also reported that the oxidative status of patients having infectious disease is very important since some pathologies arising during infection could be attributed to oxidative stress and generation of reactive oxygen species (ROS) in infection may even have fatal consequences [6,7,8]. All this allowed people in developing countries to resort to traditional medicine [9]. Indeed, the WHO estimates that 80% of the populations in Africa use medicinal plants for their health problems [10]. Natural products from medicinal plants play a considerable role in the discovery and development of new drugs [11]. In recent years, the antimicrobial and antioxidant effects of medicinal plants are receiving more attention [12,13].

* Corresponding author: Patrick Yamen Mbopi

Among these plants we find *Canarium schewenfurthii* Engl from the Burseraceae family, a tree found in several countries whose leaves and bark are used in traditional medicine to treat diabetes, malaria, infections, digestive and respiratory tract disorders [14]. This present study was carried out in the perspective of studying the phytochemical composition and the antibacterial and antioxidant properties from extracts of trunk bark of *Canarium schewenfurthii*.

2. Material and methods

2.1. Plant materials

The plant material used consisted of the bark of the *Canarium schewenfurthii* trunk. They were collected in Bangangté (Western Region) in April 2022 and identified in the National Herbarium of Cameroon at number 40804 / HNC. Our samples have been dried out of the sun at room temperature for 21 days, then sprayed with a three-phase VINCO® Model Y2-132S1-2.



Figure 1 The whole tree of *Canarium schewenfurthii*

2.2. Extraction

The extraction technique was performed according to the method described by Ozela et al in 2021[15]. 500 g of bark powder were macerated with 5 L of the ethanol-water mixture (70:30). The mixture was stirred and then allowed to stand for 48 hours. The whole was filtered with Whatman N°3 paper; then the filtrate was concentrated in a rotavapor (BUCHI R-201) under reduced pressure at 40 °C. The concentrate obtained was put in an oven at 45°C for 72 hours to obtain the dry extract which was stored at 4°C. the extraction yield was calculated according to the formula:

$$R = \frac{(\text{masse de l'extract sec})}{(\text{masse de la poudre})} \times 100$$

2.3. Microbiological material

To determine the antimicrobial activity of the extract of this plant, twenty-two bacterial strains from samples analyzed at the Microbiology Laboratory of the Cliniques Universitaire des Montagnes, purified on nutrient agar were selected. These are 22 multi-resistant isolates whose profiles are presented in Table 1

Table 1 Profile of multi-resistant bacterial isolates sought

Bacterial Isolates	Antibiotics													
	RI	AMC	FOX	CAZ	IMP	CTX	CL	CFM	SXT	LEV	GEN	CIP	VA	P
<i>E. cloacae</i>	R	R	R	R	S	R	R	R	R	R	R	R	NA	NA
<i>K. pneumoniae</i>	R	R	R	R	S	R	R	R	R	R	R	R	NA	NA
<i>P. mirabilis</i>	R	R	R	R	I	R	I	R	R	S	S	S	NA	NA
<i>Acinetobacter spp</i>	R	R	R	R	S	R	R	R	R	R	R	R	NA	NA
<i>P. luteola</i>	R	R	R	R	S	R	I	R	R	I	I	R	NA	NA
<i>E. coli productrice blse</i>	R	R	R	R	S	R	I	R	R	R	I	R	NA	NA

<i>E. hafnia</i>	R	I	I	R	S	S	R	R	R	I	S	R	NA	NA
<i>C. freundii</i>	I	I	R	R	S	I	I	R	I	S	S	R	NA	NA
<i>E. sakazackii</i>	I	I	I	S	R	R	I	I	I	S	S	R	NA	NA
<i>K. ozoenae</i>	S	I	I	S	I	I	R	R	I	R	S	R	NA	NA
<i>S. marcescens</i>	I	R	R	S	R	R	R	R	I	I	I	R	NA	NA
<i>M. morgani</i>	S	I	S	S	I	I	I	I	I	S	I	I	NA	NA
<i>E. agglomerans</i>	R	R	R	S	R	R	I	R	S	I	S	R	NA	NA
<i>S. marcescens (carba)</i>	R	R	R	I	R	R	R	R	R	I	I	T	NA	NA
<i>S. aureus</i>	I	R	R	S	R	I	I	I	I	I	S	S	S	R
<i>K. oxytoca</i>	R	R	R	R	R	I	R	R	R	R	I	R	NA	NA
<i>C. koseri</i>	S	R	R	I	S	S	I	R	R	S	S	S	NA	NA
<i>E. coli</i>	R	S	I	I	I	S	I	R	I	S	I	I	NA	NA
<i>Acinetobacter spp blse</i>	R	I	R	I	I	S	I	I	I	I	S	S	NA	NA
<i>Enterobacter spp</i>	I	I	R	R	I	S	R	I	I	R	I	S	NA	NA
<i>S. adorifera</i>	R	R	R	I	R	S	R	I	R	R	S	S	NA	NA
<i>T. guamensis</i>	R	R	R	R	R	S	R	R	I	S	S	R	NA	NA

RI : Rifampicine (05µg) ; AMC : Amoxicilline/acideclavulanique (30 µg) ; CAZ : Ceftazidime(30 µg) ; CL : Clindamycine(10µg) ; CTX : Cefotaxime(30/10µg) ; GEN :Gentaminacine(10µg) ; IMP : Imipénème(10µg) ; LEV :Levofloxacin(05µg) ; P :Pénicilline(10µg) ; SXT : Trimethoprine/Sulphaméthozaxole(25µg) ; VA : Vancomycine(30 µg) ; CIP : Ciprofloxacine (05µg) ; OFX : Ofloxacin (05µg) ; FOX : Cefoxitine (30µg) ; CFM : Cefixime(30 µg) ; NA : Not applicable ; R :Résistant ; S :Sensitive; I :Intermédiaire

2.4. Phytochemical screening

The crude extracts thus obtained were subject to the identification by precipitation reactions and staining according to the methodology of the phytochemical screening performed by respective conventional reactions [16,17]

Table 2 Usual methods of phytochemical screening

Secondary metabolite	Reagent of identification
Polyphenols	Iron III chloride to 10%
Flavonoids	Ethanol, HCl, magnésium shavings
Catechic tannin	HCl-Formalin
Gallic tannin	Iron III chloride to 2%
Saponins	Foam index
Alkaloids	Sulfuric acid, dragendorff's reagent, Mayer's reagent
Terpenoids et steroids	Methanol, chloroform, sulfuric acid and acetic anhydride
Anthraquinones	Ammonia solution

2.5. Antioxidant activity assessment

2.5.1. DPPH assay

The determination of the antioxidant power of our extract has been carried out using the DPPH radical according to the method previously described by Mbopi et al in 2021[18].

An ethanolic solution of DPPH was prepared by dissolving 4 mg in 100 mL of ethanol. Then, to 50 µL of crude extract, each, was added 950 µL of the DPPH solution. All the samples as well as the positive control (L-ascorbic acid) were

tested at different concentrations (500; 250; 125; 62.5; 31.25 and 15.62 µg/mL). The absorbance was measured at 517 nm after incubation in darkness for 30 min. The test was performed in triplicate for each concentration of samples. The antioxidant activity linked to the scavenging effect of the DPPH radical was expressed as a percentage inhibition (PI) using the formula: $PI = (1 - (A_{\text{of sample}} / A_{\text{of control}})) \times 100$. The concentration of the sample required to neutralize 50% of free radicals (IC₅₀) was obtained using Graph Pad 8 software

2.5.2. FRAP assay

The reducing power of the extract was determined by the FRAP method (Dieng et al., 2017) [19]. Thus, 0.4 ml of sample and positive control (L-ascorbic acid) at different concentrations (500; 250; 125; 62.5; 31.25 and 15.62 µg/mL) is mixed with 1 ml of phosphate buffer (0.2 M; pH=6.6) and 1 ml of potassium hexacyanoferrate [K₃Fe (CN)₆] at 1%. After incubating the mixture at 50°C for 30 minutes, 1 ml of 10% trichloroacetic acid was added to it, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1 ml of the supernatant from each tube is mixed with 0.2 ml of a 0.1% FeCl₃ solution and left to stand in the dark for 30 minutes before measuring the absorbances at 700 nm. The antioxidant activity linked to the reducing power of the extracts is expressed in Reducing Power (RP) using the following formula: $PR = 100(Aa - Ab) / Aa$

Aa: absorbance of the extract Ab: absorbance of the blank

2.6. Antibacterial activity evaluation

2.6.1. Preparation of extract solution

The extract solution was prepared at a concentration of 1000mg/mL by dissolving 3g of the extract in 3 mL of DMSO.

2.6.2. Bacterial inoculums preparation

The bacteria to be tested were subcultured onto nutrient agar in Petri dishes and then incubated for 24 hours at 37°C. The young cultures obtained were used to prepare an inoculum of opacity equivalent to the 0.5 McFarland scale, corresponding to the concentration of 1.5×10⁸ Colony Forming Units/ml (CFU/ml).

2.6.3. Determination of minimum inhibitory concentrations (MIC)

The test was performed in triplicate in sterile test tubes. 3 mL of Mueller Hinton Agar was introduced in the first tube of the range. Then, 3mL of the sterile extract solution at a concentration of 1000mg/mL were taken and introduced into the corresponding tube, followed by a series of dilutions in cascade of 2. Finally, 15 µL of bacterial inoculum were introduced into the tubes. The concentrations in the tubes ranged from 500mg/mL to 0.24mg/mL. The tubes were incubated in a water bath at 65°C to keep the agars in fusion the tubes were removed from the water bath then tilted at 45° so as to obtain a pellet and a slope, then kept for 24 hours at temperature ambient to ensure their dehydration. The MIC for each bacterial strain was thus deduced from the first tube of the range within which growth was not observed [18]

2.6.4. Determination of minimum bactericidal concentrations (MBC)

The tubes showing no visible signs of culture were reintroduced into 3 mL of sterile Mueller Hinton broth, then incubated in an oven for 24 hours at 37 °C. MBC was determined from the first tube in which there was no turbidity

3. Results

3.1. Extraction yield

For the determination of the extraction yield, the present work led to obtaining the value for this extract. Table 3 presents the details of this operation

Table 3 *Canarium schweinfurthii* extraction yield

Mass of powder (g)	Mass of dry extract (g)	Yield (%)
500	50.1	10.02

3.2. Phytochemical screening

Phytochemical analysis of secondary metabolites tested in the different extracts, allowed us to obtain the results recorded in the table 4

Table 4 Results of phytochemical analysis

Secondary metabolite		Hydroethanolic extract
Polyphenols		+
Alkaloids		+
Tannins	Catechic	+
	Gallic	-
Flavonoids		+
Saponins		-
Terpenoids		+
Steroids		-
Anthraquinones		+

(+) : Present ; (-) : Absent

3.3. Antioxidant activity assessment

3.3.1. DPPH assay

The percentages of inhibition of the DPPH radical of the L-ascorbic acid extract are presented in figure 2

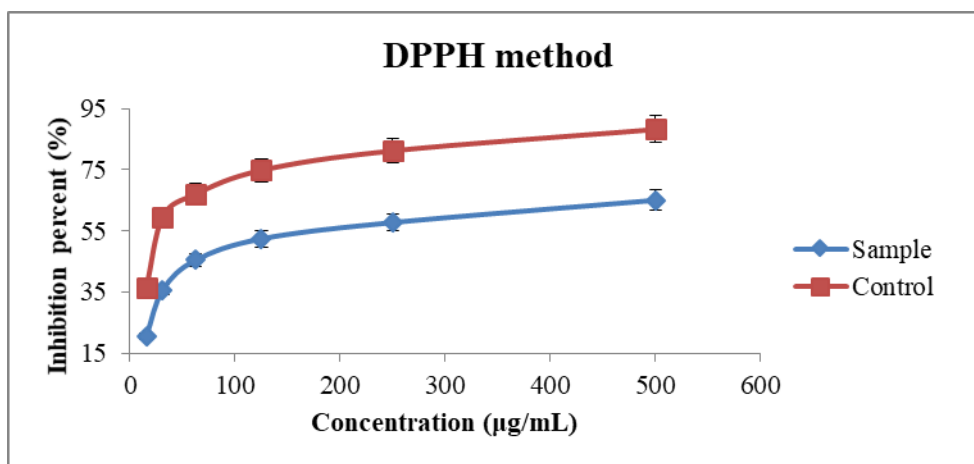


Figure 2 Percentage of DPPH radical inhibition of extract and L-ascorbic acid

From the lowest concentration, the inhibition of the DPPH radical by the extract evolves gradually until it reaches its maximum at 65.07% at a concentration of 500 µg/mL.

At all concentrations, the percentage inhibition of the DPPH radical by the L-ascorbic acid standard is greater than that of the extract.

3.3.2. FRAP assay

The percentages of inhibition of the L-ascorbic acid extract are shown in Figure 3

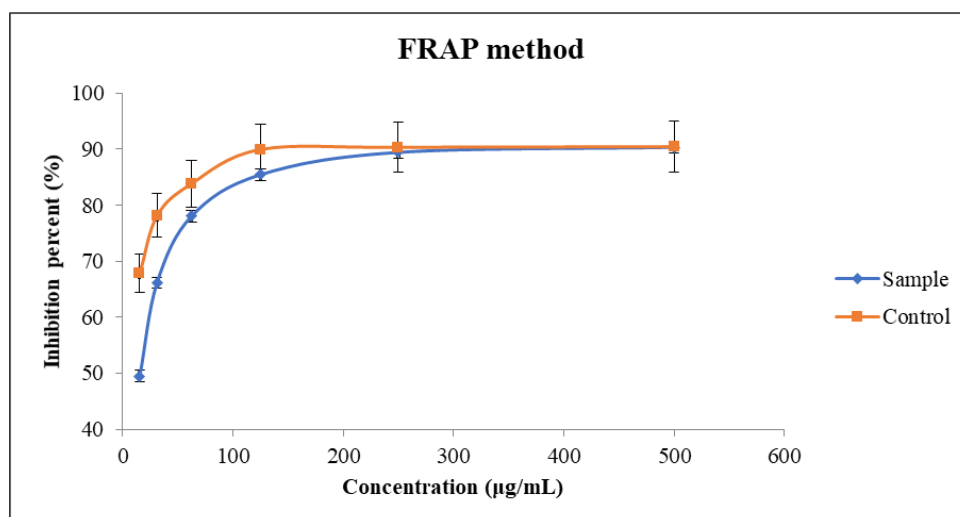


Figure 3 Percentage inhibition of extract and L-ascorbic acid

The evolution curve of the percentage inhibition of the extract evolves gradually and tends to reach a plateau from the concentration of 250 µg/mL. Concentrations ranging from 15.63 to 250 µg/mL the percentage of inhibition of the standard is higher than that of the extract then from the concentration of 250 µg/mL, they reach a plateau at the same value and no longer progress.

To make these results more explicit, the inhibitory concentration 50 (IC₅₀) of our two methods. The results are shown in Table 5

Table 5 Inhibitory concentrations 50 of the extract and the standard

Assay	IC ₅₀ (µg/mL)	
	Extract	Standard
DPPH	24,6±0,05 ^a	11,44±0,046 ^b
FRAP	8,95±0,07 ^c	5,52±0,056 ^c

Means with different letters are significantly different (P < 0.05)

The results obtained show us that the extract has a significant activity but still lower than the values of the standard.

3.4. Antibacterial activity evaluation

The results of the antibacterial activity of our extract are reported in Table 6

Table 6 Results of the antibacterial activity of the extract

Bactéria	MIC (mg/mL)	MBC (mg/mL)	R	Effects
<i>Enterobacter cloacae</i>	1,95	1,95	1	Absolute bactericide
<i>Klebsiella pneumoniae</i>	3,90	3,90	1	Absolute bactericide
<i>Proteus mirabilis</i>	3,90	3,90	1	Absolute bactericide
<i>Acinetobacter</i>	3,90	3,90	1	Absolute bactericide
<i>Pseudomonas luteola</i>	15,62	31,25	2	Bactericide
<i>E. coli BLSE</i>	7,8	15,62	2	Bactericide
<i>EntrobacterHafnia</i>	3,90	3,90	1	Absolute bactericide
<i>Citrobacter freundii</i>	3,90	3,90	1	Absolute bactericide

<i>Enterobacter sakazacki</i>	3,90	3,90	1	Absolute bactericide
<i>Klebsiella ozoena</i>	7,8	15,62	2	Bactericide
<i>Serratia marcescens</i>	3,90	3,90	1	Absolute bactericide
<i>M. morganie</i>	3,90	3,90	1	Absolute bactericide
<i>E. aggequerans</i>	1,95	1,95	1	Absolute bactericide
<i>S. marcescens</i>	7,8	15,62	2	Bactericide
<i>S. aureus</i>	1,95	1,95	1	Absolute bactericide
<i>K. oxytoca</i>	7,8	15,62	2	Bactericide
<i>C. koseri</i>	3,90	3,90	1	Absolute bactericide
<i>E. coli</i>	1,95	1,95	1	Absolute bactericide
<i>Acinetobacter BLSE</i>	7,8	15,62	2	Bactericide
<i>Enterobacter spp.</i>	1,95	1,95	1	Absolute bactericide
<i>Serratia adorifera</i>	3,90	3,90	1	Absolute bactericide
<i>Trabulsiiella guamensis</i>	3,90	3,90	1	Absolute bactericide

(R) = Ratio MBC/MIC

According to Ndouguet et al in 2021, when $R < 4$ it is said to be bactericidal and when $R \geq 4$ it is said to be bacteriostatic [16].

The MICs obtained vary between 1.95 (*Enterobacter cloacae*, *Enterobacter aggequerans*, *S. aureus*, *Enterobacter spp*) and 15.62 mg/ml (*Pseudomonas luteola*). As for the MBC, the values vary between 1.95 (*Enterobacter cloacae*, *Enterobacter aggequerans*, *S. aureus*, *Enterobacter spp*) and 31.25 (*Pseudomonas luteola*). The table 6 also shows a strong bactericidal effect on the strains tested with a predominance of absolute bactericidal.

4. Discussion

The aim of this work was to determine the antibacterial and antioxidant potential of the hydro-ethanolic extract of the trunk bark of *Canarium schwenfrthii* on multi-resistant strains expressing some known resistance mechanisms. The choice of the study being made with a view to contributing to the enhancement of products made from plants used in traditional medicine in Cameroon.

The yield of the extraction was 10%, this yield would justify the use of the solvent for the preparation of phytomedicines. Indeed, the mixture of ethanol solvent and water would make it possible to extract polar compounds from the plant [19]. Moreover, this yield is similar to the yield obtained in the work of Nzodjou et al in 2023 [20] who had used the same solvent mixture. On the other hand, the yield was very low (2.29%) in the work of Teinkela et al in 2022 [21]. This low yield can be justified by the use of ethanol in their work.

Six of the nine secondary metabolites sought were found, in particular polyphenols, flavonoids and tannins, which are polar compounds. The work of Nzodjou et al in 2023 and Teinkela et al in 2022 also made it possible to find the same secondary metabolites. This result would further show that ethanol as extraction solvent allowed to extract the same compounds as with the ethanol-water mixture. But the latter extracted the compounds in quantity which justifies the extraction yield of 10% in our work.

The antioxidant activity was carried out by two methods, namely that of DPPH and that of FRAP. The antioxidant activity of the hydro-ethanolic extract showed inhibitory activity against the free radical DPPH ($IC_{50} = 24.6 \pm 0.05 \mu\text{g/mL}$) and the ferric ion ($IC_{50} = 8.95 \pm 0.07 \mu\text{g/mL}$) but this inhibition remains slightly weak compared with the standard used ($IC_{50} = 11.44 \pm 0.046 \mu\text{g/mL}$; $5.52 \pm 0.056 \mu\text{g/mL}$). Our extract showed better antioxidant activity with results close to the standard in the FRAP method. The work of Wahab et al in 2015 showed inhibitory concentrations 50 of around 17.28 to 48.15 $\mu\text{g/mL}$ for the DPPH test and 106 to 358.96 $\mu\text{g/mL}$ for the FRAP method [22]. This could be justified by the use of the fruit of *C. schwenfrthii* and fractions from partitions with different solvents.

The analysis of the results of the antibacterial activity of the extract showed that our extract has antibacterial potential on all proven multi-resistant bacterial isolates with MIC values varying between 1.95 mg/mL to 15.62 mg/mL and CMBs from 1.95 mg/mL to 31.25 mg/mL. These results also indicate that the MIC and CMB values vary very little with an absolute bactericidal potential on multiresistant bacteria and those which express resistance to conventional antibiotics by the expression of low-level cephalosporins, broad-spectrum beta-lactamases and penicillinases. The bactericidal potential of this plant was also reported on bacteria and yeasts by Obame et al in 2007 [23] who had worked on the essential oil of *C. schwenfrthii*

However, these antibacterial activities would be linked to the major metabolites contained in the extracts. Indeed, phenols are recognized as modulators of the intestinal bacterial flora and the preservation of fruits, therefore, inhibitors of microbial growth. In this sense, they would reduce bacterial metabolism (bacteriostasis, or bactericide) [24]. However, according to a classification of the activity of plant extracts according to the value of their MIC revealed by Toam et al in 2016 [25], our extract used in this study is weakly active on all bacterial strains. Indeed, for this author, the activity of an extract is important if the MIC is less than 500 µg/mL. It is said to be moderate if the MIC is between 500 and 1500 µg/mL and low if the MIC is greater than 1500 µg/mL [25].

5. Conclusion

Hydroethanolic maceration of the trunk bark of *C. schwenfrthii* is an interesting technique for the production of active extracts. The result of this study highlighted that the antibacterial, antioxidant activities demonstrated by the hydroethanolic extract *in vitro* are due to the presence of bioactive compounds. Additional research is needed to identify therapeutic molecules or even to study the *in vivo* efficacy to allow the production of Phytomedicines.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicting interests.

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