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Antioxidant and Ethnobotanical Study of *Strychnos icaja* Baillon (Loganiaceae) from Gabon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RRRAS, SAA and LEM performed the experimental studies and drafted the manuscript. Authors RRRAS and SAA played roles in the writing and editing of the manuscript. Author SAA conceived of the study, participated in the design and coordination of the study, supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: In plants, are found large amount of vitamin (A, B, C, E), mineral (Zn, Cu, Se) and biochemical (glutathione, taurine, phenol acids) substances which also have a well-known antioxidant power. These substances have phytotheraptic applications in the fight against aging caused by oxidative stress and are oxidative component of acute pathologies: (infectious, traumatic, inflammatory and allergic). *Strychnos icaja* Baillon (Loganiaceae) is a tropical shrub common in the tropical forest of Central Africa. *Strychnos* species is mainly used by the local population as an arrow or ordeal poison, for treatment of haemorrhoids and malaria by the Pygmies tribes from Cameroon due to its magic-medical property. This study determined the medicinal uses of *Strychnos* and assessed its antioxidant potential.
Methodology: Extracts of alkaloids, aqueous, ethyl acetate, dichloromethane and butanol from *Strychnos* were evaluated for their antioxidant properties. An ethnobotanical survey of 20 traditional therapists was carried to obtain the medicinal uses of the root of *Strychnos icaja*.
Results: 1,1-diphenyl-2-picryl-hydrazyl (DPPH) test shows good antiradical activity. Antioxidant

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activity by the ferric reducing antioxidant power (FRAP) test, show that *Strychnos* total alkaloids of the roots have a value of 5.94 ± 0.14mmol EAA/g that is significantly close from Ascorbic acid (5.86 ± 0.51 mmol EAA/g). The results of survey of traditional therapists identified six indications of this plant, namely in the treatment of haemorrhoids, against ear infections, male urethritis, rheumatic and dental pain and sterility in women.

Conclusion: The *Strychnos icaja* possesses antioxidant activity, this can find use in the treatment of inflammatory diseases.

Keywords: Strychnos icaja; antioxidant; medicinal plant; inflammatory diseases; free radicals; oxidative stress.

1. INTRODUCTION

Oxidation is a phenomenon linked to life itself: oxidation provides the energy necessary for our cells and to defend themselves against external aggressions. However, it is the combination with oxygen that causes the appearance of free radicals in our body, unstable molecules greedy for electrons and which cause biochemical chain reactions. These free radicals, so useful because they allow the homeostasis of the organism, at the same time cause oxidative stress which inexorably causes the aging of this organism. Gabon is located astride the equator. It has an area of 267, 667 km² with a forest that covers 80% of the territory. This forest contains many species that are used by people in therapy to prevent or cure certain conditions [1]. The majority of these people had faith in traditional medicine, some people use it in any exclusivity while others adopt in addition to modern medicine. It concerns the treatment of common ailments and has an alternative to the failures of modern medicine. In addition to these uses, many attribute magical properties to certain medical-species which are used not only during the various ceremonies rite (marriage, bereavement, initiation, etc.) but also to treat conditions such as spells, bad eye, etc. In general, traditional medicine is used today as it once was, despite the rise of modern medicine, and especially since the emergence of' ''so-called incurable diseases.'' Hence our interest in *Strychnos icaja* is a plant commonly used by people who attribute properties therapeutic and magic [2]. The *Strychnos icaja* Baillon, is known by the vernacular names: Ikaza (Mpongwè), Ikadja kwaï (Benga), Kasé (Bakèlè), Mbundu (Galoa, Nkomi, Oroungu, Ngowé, Eshira, Bavarama, Bavoungou, Bapunu, Balumbu, Bavili, Baduma, Banzabi, Loango, Masangu, Mindumu), Mbondo (Ivea, Bavové, Bakota), Molela (Apindji), Mvya (mitsogo), Bilon (Fang) [3]. It belongs to the Loganiaceae family, is a liana that can reach a height of 20-40 m and a length of 20-100 m with a stem of 4-15 cm in diameter. The *Strychnos icaja* Baillon occurs in the forests of Central Africa (Congo, Cameroon, Rwanda, and Gabon). It is a plant of dense forests and secondary [4]. Root barks are used as an arrow poison by some pygmy tribes of Cameroon. This toxicity was attributed to the strychnine and hydroxystrychnine mono-indole alkaloids isolated from the roots by Sandberg et al. [5,6,7]. They are also used in the treatment of malaria. Root barks are used in sitz baths against haemorrhoids. Dry root bark powder, mixed with palm oil, is applied topically to affected areas in dry or oozing skin dermatoses [8]. It would be aphrodisiac at low doses. It is also used as an emetic, to '' wash the belly '' and against ear infections. In addition to its therapeutic use, the root is frequently used by populations for its pharmacological properties. The macerated root rasp is used in the treatment of measles, the decoction of liana against beriberi, the calcination of leaves against back pain and diabetes, the decoction of leaves and roots against epilepsy [9]. *The Strychnos icaja* been the subject of several studies, particularly on alkaloids; vomicine; 19, 20-α-epoxinovacin; 19,20-α-epoxy, 15-hydroxynovacin; strychnine; isostrychnine; bisnordihydrotoxiferrin: sungucine; isosungucine; 18-hydroxysungucin; 18-hydroxyisosungucin were isolated from the roots of Strychnos icaja from Congo [10,11]. These compounds, in particular 18-hydroxy isosungucine, are moderately active against Plasmodium falciparum. Another compound, Strychnogucin B is cytotoxic against the human KB cell line of cancer and human fibroblasts WI38 [12]. Lusakibanza M et al [13] have also shown that the methanolic and dichloromethane extracts of the root bark had a very good selectivity index on falciparum strains. In addition to this interesting antimalarial activity, it showed moderate in vitro antitrypanosomal activit [14]. We know that free radicals are responsible for degenerative diseases like Alzheimer and Parkinson's or chronic diseases like diabetes,

cancer, inflammatory diseases. Thus we hypothesize that this plant which has demonstrated anti-cancerous properties and which is used in the treatment of epilepsy by traditional therapists could have antioxidant properties. Our work aims are to determine medicinal uses of *Strychnos* and to assess its antioxidant potential.

2. MATERIALS AND METHODS

2.1 Plant Materials

Plants were harvested in the forest of the Cap Mondah Esterias (latitudes: 0°35.33' N; longitudes: 9°20.27' E 46 m); Gabon. The harvest was conducted between January and March 2020. Identification of plant material has been made on the ground and the National Herbarium of Gabon. Drying takes place in the shade at room temperature for 2-3 weeks. A voucher specimen was deposed in this department (Maesen et al. 5907; A.J.M. Leuwenberg.11470; J.M.et B. Reitsma.2137; R. Sita.5172).

2.2 Data Collection

Surveys were conducted on three cities: Libreville, Lambarene and Cap Esterias. In total 20 traditional therapists and users were surveyed and interviewed based on the current medicinal uses of Strychnos.

2.3 Preparation of the Aqueous Extract

The spraying was done after drying the harvested plants. We used a flail chopper (Model SK 100) for the coarse powder. Samples were taken from the entre plant. The root, stem and leaf samples were crushed separately. The aqueous extract of the root where obtained by macerating 100 mg of root in 500 ml of water during 24H. After filtration the marc is returned two more time. Filtrates were collected and frozen (Laborota 4002-Control Heidolph, Germany), then lyophilized.

2.4 Preparation of Extracts by Solvents of Increasing Polarity

About 50 g of *Strychnos icaja* roots powder is macerated for 24 hours with acetone/water (80:20). After filtration, acetone is removed in a rotavapor at 40°C. The aqueous extract is taken with butanol and shook in a funnel. Then the butanol fraction is evaporated in a rotavapor at 40°C to obtain the butanol fraction. After that, the residue is shaken again with dichloromethane and evaporated to obtain the dichloromethane fraction. Finally, the last residue is shaken with ethyl acetate and evaporated to obtain the ethyl acetate fraction.

2.5 Alkaloids Extraction

The drug used is the stem and root powder for all tests. Introduce 100 g of dry, coarsely pulverised drug into a brewer. Add dilute sulfuric acid (1: 20 concentrated H2SO4 with distilled water) in a ratio of 5: 1 to 10: 1 (volume of acid: the weight of the plant) and then stop. Stir and let macerate for 24 hours at laboratory temperature. Filter on paper and wash with water to obtain about a filtrate. Then alkalinise the filtrate with ammonia to pH 8-9 and put in a separatory funnel. Add chloroform and stir without emulsification. After decantation, remove the organic phase. Repeat this operation until the alkaline solution no longer precipitates with the Dragendorff reagent. Combine the organic phases and dry over anhydrous sodium sulphate, filter and transfer to a calibrated evaporation flask to dry rotavapor under reduced pressure.

2.6 Reducing Powered with FRAP Method

The FRAP method (Ferric Reducing Antioxidant Power) is based on the ability of extracts to reduce ferric ion (Fe3+) by ferrous ion (Fe2+). Total antioxidant capacity of each plant extract was determined by the method Benzie and Strain [15]. And 1 ml of an aqueous solution of each extract (10 mg/ml diluted 100th for 0.1 mg/ml), ascorbic acid, was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of the aqueous solution (1%) of potassium hexacyanoferrate [K3 Fe (CN) 6]. After 30 min of incubation at 50°C, 2.5 ml of trichloroacetic acid (10%) was added. The mixture was then centrifuged at 3000 rpm/min for 10 min. 2.5 ml of the supernatant were then mixed with the same volume of water, and 0.5 ml of a freshly prepared aqueous solution of FeCl3 (0.1%) was added. The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-100 mg / l). The reducing power is expressed in ascorbic acid equivalent (AAE) (mmol ascorbic acid / g of dry extract).

2.7 Antiradical Activity by the Method of Inhibition of DPPH Radical

The antiradical activity of plant extracts reflects their ability to scavenge free radicals from the body. The radical scavenging activity was

evaluated on the different fractions of Strychnos icaja. The extracts were dissolved in methanol to obtain concentrations of mothers of 10 mg/ml. This concentration is diluted to 100 for the second test. The method spectrophotometric 2.2-diphenyl-1-picrylhydrazyl (DPPH) as described by Popovici et al. [16] is used with some modifications. Introduce 1.5 ml of a methanol solution of DPPH at 20mg / l in test tubes containing 0.75 ml of extracts prior to the test. A control containing no plant extract is also prepared. The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-200 mg / l). Each test was performed in triplicate. Antiradical power was expressed as ascorbic acid equivalent (AAE) (mmol ascorbic acid / g of dry extract). The concentration of reducing compounds (antioxidants) in the extract is expressed in mmol ascorbic acid equivalent (AAE) / g of dry extract.

2.8 Antiradical Activity by the Method of Inhibition of the Radical Cation ABTS

The method described by Pellegrini et al. [17] is used. It is based on the discolouration of a stable radical cation, ABTS + (2.2 '-azinobis- [3-acid-6-sulfonic ethylenzothiazoline]') to ABTS in the presence of antioxidant compounds at 734 nm. The radical cation ABTS + was generated by reacting an aqueous solution of ABTS (7 mM) with 2.5 mM potassium persulfate (final concentration), the mixture is kept in the dark at room temperature for 12 hours before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 to 734 nm using the spectrophotometer. For each extract, a methanol solution (10 mg/ml) is diluted to 100th in ethanol µl of sample and 10 (solution), the reference substance (ascorbic acid) was mixed with 990 µl of a fresh solution of ABTS +. The set is stored away from light for 15 minutes and absorbances were read at 734 nm in a spectrophotometer against a standard curve of ascorbic acid precisely 6 min after initial mixing. The concentration of compounds having a reducing effect on the radical cation ABTS + is expressed in mmol ascorbic acid equivalent (AAE) / g of dry extract. The concentration of reducing compounds (antioxidants) in the extract is expressed in mmol ascorbic acid equivalent (AAE) / g of dry extract.

2.9 Statistical Analysis

All data represent the average of three trials. For the comparison of the results, analysis of the variance, ANOVA and Dunnett test (GraphPad Instat) are used and the degree of data significance is taken at 95% level of probability ($P \leq 0.05$).

3. RESULTS

3.1 Ethnobotanical Survey

Interviews with some traditional healers (January 2020 to March 2020 in Libreville, Lambarene and Cap Esterias) were conducted to allow us to know the extent of uses of *Strychnos icaja* against haemorrhoids and most important. The *Strychnos icaja* has been used to as plant designated to prevent harmful influences of witches and other spirits bad. All those who use it for its magic properties are unanimous about the almost total protection which ensures they feel secure so they put root at the entrance to their home as the top of the door, or they bury it across their concession or put it in their car and the other more suspicious focuses on them as a talisman. For them, the plant represents the original judge, is the plant which since time immemorial has the power to judge men and to punish. *Strychnos icaja* is found in the deep forest. It is very difficult to harvest the vine because once she wraps the top of tall trees, it is possible to recognize it unless it has a branch which is still under a shrub. The *Strychnos icaja* users say it must pronounce certain words and talk to the plant when the roots are dug up if you want to avoid it breaks. It is indeed true that the orally administered itself Mbundu people suspected of witchcraft, but in certain rituals had to dig up the root without it breaks if they were considered unclean. During our interviews, the traditional healers interviewed showed a lot of reluctance. The only use they were willing to disclose was that of protection. During our visit to the markets, we found stalls where many roots were exposed. Nevertheless, despite the difficulties encountered, we were able to obtain some recipes: in the treatment of haemorrhoids, against ear infections, male urethritis, rheumatic and dental pain and sterility in women (Table 1).

3.2 Antioxidant Activities by Inhibition of DPPH

Antiradical activity by the method of inhibition of DPPH radical has a content of 13.33 ± 0.16 mmol AAE/g for St (*Strychnos*) total alkaloids roots; 12.82 ± 0.10 mmol AAE/g for St dichloromethane; 12.97 ± 1.05 mmol AAE/g for

Table 1. Recipe in which Strychnos icaja is used obtained from traditional therapists

Recipe 1	Family	Use
Treatment of hemorrhoids Part used: root barks Product added : Kaolin (red clay)		The root barks were mixed with the Kaolin previously moistened with water. We realize suppository and they are dried. Used daily, the disease disappears after one week.
Recipe 2 Treatment of otitis Part used: root bark Product added: Banana leaf		The bark of roots are steeped in water a few moments. They are then recovered and introduced into a funnel made with a piece of banana leaf previously spent on the fire. We put a few drops in the ear until healing. NB: You can use other leaves instead of banana leaves.
Recipe 3 Dental bread Part used: root bark Product added: Capsicum annuum L.	Solanaceae	Crush the pepper and mix with the root bark then apply the mixture on the decayed tooth.
Recipe 4 Treatment of urethritis in men or that does not cure (according to the local expression) Part used: internal part of the grated bark Product added : - *Aframomum melegueta* K.Schum: 5 seeds, powdered - *Citrus limon (L.) Osbeck*: lemon juice	Zingiberaceae Rutaceae	Mix well and let stand an hour or two, then filter for a liquid without solid particles. The liquid is to be administered once in intra-urethral injection with a syringe (without needle). It's very painful for the patient, but treatment would cure urethritis with the evacuation of more or less solid debris (stripping)
Recipe 5 Rheumatic Pain in osteoarthritis Part used: grated bark Product added : - *Solanum mammosum*: fruit - *Solanum torvum*: fruits and leaves - *Struchium sparganophorum* (L.) Kuntze: stems + leaves - *Acanthus montanus (*Nees) T.Anderson: leaves NB: This is also here to scarify grass. - *Citrus limon (L.) Osbeck*: lemon fruit juice. - *Costus lucanusianus* J.Braun & K.Schum: crushed juice of the stem	Solanaceae Solanaceae Compositae Acanthaceae Rutaceae Costaceae	The remaining water after rain in the hollow of a tree trunk or a rock. Sweeps of bark, leaves, stems, fruits are crushed and mixed with finely enough lemon juice and the juice of *Costus* in a pot. Scarification is done on the diseased areas (joints or others) with a branch of Acanthus which one whips the skin and one apply morning and evening this paste until the improvement of osteoarthritis pain. NB: In the past we used as pot the fruit of *Strychnos aculeata* (Loganiaceae)
Recipe 6 Difficulty giving birth Part used: grated root bark in tiny doses Product added: *Buchholzia coriacea* Engl. (Onion gorilla): fruit	Capparaceae	The bark is crushed with onion gorillas, add water, then let macerate filtered and administers enemas

St ethyl acetate; 12.80 ± 0.04 mmol AAE/g for St butanol; 5.21 ± 0.01 mmol AAE/g for St aqueous root extract and 13.76 ± 0.26 mmol AAE/g for quercetin (Table 2).

3.3 Reducing Powered with FRAP Method

Reducing powered with FRAP method has a content of 5.94 ± 0.14 mmol AAE/g for St total

alkaloids roots; 2.78 ± 0.10 mmol AAE/g for St dichloromethane; 2.81 ± 0.01 mmol AAE/g for St ethyl acetate; 2.16 ± 0.06 mmol AAE/g for St butanol; 0.46 ± 0.27 mmol AAE/g for St aqueous root extract and 5.86 ± 0.51 mmol AAE/g for ascorbic acid (Table 2).

3.4 Antiradical Activity by Inhibition of ABTS +

Antiradical activity by the method of inhibition of the radical cation ABTS + has a content of 6.74 ± 0.14 for St total alkaloids roots, 3.83 ± 0.15 for St dichloromethane, 3.77 ± 0.20 for St ethyl acetate, 2.24 ± 0.57 for St butanol, 0.86 ± 0.25 for St aqueous root extract and 1.78 ± 0.58 for ascorbic acid (Table 2).

4. DISCUSSION

Strychnos icaja Baillon belongs to the Loganiaceae family. It is a vine reaching a height of 20 to 40 m and a length of 20 to 100 m. It is found in the forests of Central Africa. This Strychnos is used in the past by the populations as an arrow poison or test. During our investigation most traditional healers agreed to recognize the plant as a protective power against certain practices of witchcraft. We were able to obtain some recipes from some traditional healers and users of the plant, namely: against otitis, male urethritis, rheumatism, dental pain and sterility in women. Previous work carried out on the upper plateau of whereby Frederich et al, [11] only the alkaloids of the roots from which isosungucin, 18-hydroxyisosungucine, pseudo strychnine and many other alkaloids were isolated. They also carried out antimalaria tests and found that strychnogucin had a good inhibitory concentration against Plasmodium falciparum (0.617 µm), which constitute ~~is~~ almost half that of quinine (0.269 µm). Denoel et al, [18] found alkaloids in barks of stems, roots and leaves.

The use of this plant against dental pain can be explained by the analgesic properties of the alkaloids [19]. Many authors have shown that medicinal plants have excellent anti-inflammatory properties. This is the case of *Curcuma longa* [20], *Carica papaya* [21]. The existence of tannins in the plant could explain its use against haemorrhoids and cutaneous dermatoses especially that the tannins have the property to agglutinate [22]. From the extracts have been found antimicrobial, antiviral and hypoglycemic properties [23]. Besides the tannins, there are flavonoids. The presence of flavonoids could justify their venotropic properties (against varicose veins and haemorrhoids) [23]. These compounds arouse great interest due to their numerous beneficial uses for human health: their antibacterial, antiviral, antiplatelet, antiallergic, anti-inflammatory, anti-tumor properties and their antioxidant activities are the subject of in vitro and epidemiological studies with in a the therapeutic goal in the treatment of certain cancers, inflammatory, cardiovascular and neurodegenerative diseases [24,25,26]. Some of them are also used as additives in food, pharmaceuticals and cosmetics [27,28,29].

The second part of our work consisted of evaluating the antioxidant activity of *Strychnos*. This was done to enrich the body of information and documentation of the antioxidants concentrations and use to mitigate the inflammatory processes. This activity has been

Table 2. Evaluation of the antioxidant activity of *Strychnos icaja* extracts in comparison with quercetin and ascorbic acid by different methods

Extract type	DPPH (mmol AAE/g)	FRAP (mmol AAE/g)	ABTS (mmol AAE/g)
St alkaloids total roots	13.33±0.16	5.94±0.14	6.74±0.14**
St dichloromethane roots	12.82±0.10	2.78±0.10**	3.83±0.15**
St ethyl acetate roots	12.97±1.05	2.81±0.01**	3.77±0.20**
St butanol roots	12.80±0.04	2.16±0.06**	2.24±0.57
St extracted aqueous roots	5.21±0.01**	0.46±0.27**	0.86±0.25
Quercetin	13.76±0.26	Nd	Nd
Ascorbic acid	Nd	5.86±0.51	1.78±0.58

Values are expressed as mean ± SE of three replicates
*NB: ** p <0.01: the difference is very significant*
** p <0.05: the difference is significant*
Nd: not determined
St (Strychnos)

ascribed to the phenolic compounds present in it, particularly flavonoids and tannins [30,31,32,33]. Antiradical activity by the method of inhibition of DPPH radical shows that St alkaloids root and St alkaloids stem have the best inhibition of radical DPPH followed with St dichloromethane, St ethyl acetate, St butanol and much less for St root aqueous extract. Those values except St root aqueous extract are significantly close to that quercetin (13.76 ± 0.26 mmol AAE/g). Reducing powered with FRAP method show the same result, with high value to St alkaloids root and low value to St aqueous extract compared to ascorbic acid (5.86 ± 0.51 mmol AAE/g). The FRAP test makes it possible to evaluate the antioxidant power of foods by determining this capacity for reducing ferric ions to ferrous ions. The antioxidant content is determined by the solutions containing the known concentrations of ferrous ions. FRAP is expressed in mmol of antioxidants per 100 g of food. Here is how to interpret the FRAP values of foods: 0 <FRAP index <1.5: low antioxidant capacity; 1.5 <FRAP index <3: average antioxidant capacity; 3 <FRAP index <10: high antioxidant capacity; FRAP index> 10: very high antioxidant capacity [34]. The alkaloids total in the roots have a high antioxidant capacity and St aqueous extract a low antioxidant capacity extract. Antiradical activity by the method of inhibition of the radical cation ABTS show high value compared to ascorbic acid reference substance (1.78 ± 0.58 mmol AAE/g). Free radicals are harmful substances generated by the body that damage cells, causing premature ageing and the development of certain diseases. Thus, the role of antioxidants is to combat the oxidation caused by these free radicals. However, they are more or less held in check by the body's natural antioxidants. Some factors can break this balance. If free radicals come to exceed the body's ability to neutralise them, they can contribute to the onset of many diseases, including cardiovascular disease, certain types of cancer and other diseases associated with ageing [35]. Lansiaux et al. [36] have isolated from *Strychnos icaja* root, and a compound named Strychnogucin B, who's cytotoxic against the human KB cell line of cancer and against human fibroblasts WI38. Many chemicals in foods are called antioxidants because they have the property of preventing harmful chain reactions caused by free radicals. They are bulletproof for the body. The main natural antioxidants are bioflavonoids, carotenoids, vitamins C and E, and selenium [37]. Compared to quercetin or vitamin C, our extracts have excellent antioxidant activity. Aworet and al., [38] have shown that *Strychnos icaja* contains phenolic compounds. These results are consistent with what is reported in the literature by several authors that the potential for antioxidant activity of an extract depends on its content of phenolic compounds [39,40,41,42,43]. The dosage of this plant confirms that it is rich in phenolic compounds which are able to trap free radicals and therefore reduce oxidative stress.

5. CONCLUSION

Our study aimed to identify the traditional uses of *Strychnos icaja* antioxidant power. The results of the survey showed that *Strychnos icaja* is frequently used by local populations (in town as well as in village) due to its magical properties and in the treatment of various ailments, despite its toxicity. The findings illustrates the case of plants deemed to be toxic and whose uses are mastered by traditional therapists, and the pharmacological analysis confirmed therapeutic properties of the plant. Given the uses of this plant by traditional therapeutics and populations, it would be interesting to prevent the perennially of the species to introduce it within our arboretum.

CONSENT

As per international standard or university standard, participant's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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