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In Vitro study of the nutritional and pharmacological contribution of *Pennisetum purpureum* Schumach leaves: Natural food used by guinea pig (*Cavia porcellus*) breeders in Gabon

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Abstract

Pennisetum purpureum is a natural food used by guinea pig (*Cavia porcellus*) farmers in Haut Ogooué, Gabon. The objective of the present study was to investigate the nutritional and pharmacological contribution of *Pennisetum purpureum* Schumach leaves. The determination of the nutritional value was done by the determination of proteins, total sugars, lipids, dry matter, ash, vitamins (vitamin A, vitamin C and vitamin B6) and minerals (iron, phosphorus, nitrogen). Determination of pharmacological properties was carried out by testing for antioxidants (DPPH radical scavenging capacity measurement), egg albumin protein denaturation and antibacterial properties (diffusion method). The results show that the leaves of *Pennisetum purpureum* Schumach have high moisture, crude cellulose, lipids, proteins and ash, while total sugars and citric acid are low. The plant studied contains sufficient phosphorus and iron. Vitamins A and B6 are in reasonable amounts. *Pennisetum purpureum* Schumach has a low antioxidant activity. Anti-inflammatory activities were found to be low, with an IC50 of 65.047 µg/mL. The extracts showed maximum relative percentage inhibition against *Staphylococcus aureus* ATCC25293 BHI (100%), *Escherichia coli* 105182 CIP (111.64%), *Staphylococcus aureus* (112.5%) and *Salmonella typhi* (81.25%). In sum, the presence of nutritive and pharmacological elements could justify its use in the breeding of guinea pigs in Gabon.

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Introduction

Pennisetum purpureum Schumach (*P. purpureum*) is a species of perennial grass cultivated in dry or humid conditions, small-scale or large-scale agriculture. *Pennisetum purpureum* is native to tropical Africa, where it is called elephant grass, a favourite food of elephants. The species has been introduced into most tropical and subtropical countries as fodder for livestock (Mannetje, 1992; FAO, 2015).

P. purpureum has a vigorous root system, which develops from the nodes of its creeping runners. *P. purpureum* grass (Elephant grass) forms thick, dense clumps, up to 1 m in diameter. The leaves are flat, linear, hairy at the base, up to 100-120 cm long and 1-5 cm wide, bluish green in colour. The leaf margin is finely toothed and the blade has a prominent midrib (Françis, 2004). Elephant grass closely resembles sugarcane (*Saccharum officinarum*) but has narrower leaves and taller stems (DAF, 2022).

P. purpureum is suitable for feeding cattle and buffalo and for African elephants (FAO, 2015). The young leaves and shoots are edible by humans and can be cooked to make soups and stews (Burkill, 1985). In Gabon, several farmers use the leaves of Pennisetum purpureum Schumach. to feed guinea pigs (Cavia porcellus). The genus Pennisetum contains remarkable natural bioactive components and is frequently used for therapeutic purposes such as the treatment of dysentery, fever, diabetes and abdominal pain (Ojo et al., 2022). Previous studies have shown that P. purpureum has some biological (Ujang et al., 2021; Han et al., 2022) and digestibility properties (Wahyono et al., 2022). Very few studies have been carried out on the anti-inflammatory, antioxidant and antimicrobial activities of P. purpureum leaves. Given the importance of the food for guinea pig rearing in the province of Haut Ogooué, the nutritional and pharmacological contribution of P. purpureum leaves was studied in this work.

Methods

Collection of the plant

P. purpureum (Acc. No. 20220810) was collected in

the periphery of the University of Science and Technology of Masuku (Franceville-Gabon) during the month of November 2021 and the identification was confirmed by a botanist of the University of Science and Technology of Masuku. The *P. purpureum* leaves were selected because of their dietary importance in guinea pigs.

Determination of moisture content

The moisture content (Th) was determined after oven drying until the sample mass was stabilised. The moisture content was determined as a percentage by the ratio:

$Th = (M_1 - M_2) / M_1 \times 100$

with M_1 : fresh mass of the leaves; M_2 : mass after drying in the oven.

Determination of the protein content

The protein content was characterised by the method of Bradford (1976). It consisted of finely grinding 0.625g of *Pennisetum purpureum* leaves in the presence of 0.25 mL of the extraction solution (prepared from SDS, β -mercaptoethanol and distilled water). The grind was then centrifuged for 10 minutes (5000 G) and 0.1 mL of the supernatant was mixed with 0.9 mL of distilled water and 1 mL of Coomassie Blue previously prepared in perchloric acid solution. The absorbance was read at a wavelength of 620 nm. Bovine serum albumin (BSA) was used as a calibration and the resulting straight-line equation was used to determine the protein concentration. The protein level is expressed as a percentage by the following calculation:

Protein level (g/100g) = [(f) x(V) x(q)]x(m-1), where: f is the dilution factor; q is the mass concentration of protein in the sample (μ g/mL); V is the total volume of filtrate after centrifugation (mL); m is the test portion (g).

Determination of lipid content

Fat was extracted by the soxhlet method. A 5 g sample of leaf powder from each plant (M) was placed in a pre-weighed Wattman cartridge which was then placed in an extractor. A flask containing 250 mL of

hexane (M_1) was connected to the heated system on a selecta cap. Four hours later, the flask was removed from the soxhlet and the solvent was removed on a Janke and Kunker-RVo5-ST rotary evaporator. The flask was then dried in an oven at 80 °C for 24 hours and weighed (M_2) . The fat content was calculated according to the following formula:

Fat content (%) = $[(M_2 - M_1)x(M-1)]x_{100}$.

Determination of total sugars

The sugar content of *P. purpureum* leaves was determined by the Dubois method (Albalasmeh et *al.*, 2013). This method allowed the determination of oses using phenol and concentrated sulphuric acid. In the presence of these two reagents, the sugars give a creamy yellow colour, the intensity of which is proportional to the concentration of total sugars. The optical density is determined at 490 nm.

Determination of the crude ash content

The total mineral content was determined from a 5 g test sample. The test sample was placed in a porcelain crucible which had been heated to 550°C, cooled in a desiccator and tared. The whole assembly is gradually heated to 550°C in a furnace for slow carbonisation without ignition. The temperature is thus maintained at 550°C for 6 hours and white ash is obtained.

The ash content (Tc) was calculated as follows:

Tc(%) = 100 (Mi - Mf)/Me where, Mi = mass of the empty calcined crucible; Mf = mass of the calcined crucible + ash and Me = mass of the test sample.

Determination of crude cellulose

Crude cellulose was determined by the Sheerer method (Thiam et *al.*, 2021). For this method, 1 g of dry leaves was mixed with 50 mL of Sheerer reagent (previously prepared from 28 g of trichloroacetic acid dissolved in 700 mL of glacial acetic acid, 300 mL of distilled water and 68 mL of concentrated nitric acid).

The mixture was boiled for 30 min until cellulose was obtained. After washing with petroleum ether and filtration under vacuum, the filtrate was heated in an oven for 24 hours at 100 °C. Subsequently calcination

With : M'o: mass of dry leaf; M'1: mass obtained after oven drying; M'2: mass obtained after calcination.

Determination of the citric acid equivalence

The citric acid equivalence was characterised by neutralising the sample extract with NaOH in the presence of 1% phenolphthalein. This method is used by Marier and Boulet (1958), in fact 5g of dry leaves previously crushed mixed with distilled water (50 mL) and a drop of 1% phenolphthalein, were titrated with 0.1 N sodium hydroxide until the pink colour changed. The citric acid equivalence expressed as a percentage was determined by the following ratio: $A = (V_{NaOH} \times N \times V_1 \times 0.064) /m \times V_2 \times 100$

With: A = % citric acid equivalent in fresh material; V_{NaOH} = Number of mL of NaOH used for titration; N = Normality of NaOH (0.1N); V₁ = Total volume of filtrate (mL); V₂ = Volume of titrated extract in mL; 0.064 = Weight of one milliequivalent citric acid; m = Mass of ground sample (g)

Determination of vitamins Vitamin A level

The determination of vitamin A content was carried out by the method of Strohecker et *al.* (1965), according to which 0.5 g of leaf sample was mixed with 5 mL of ethanol (95%) in 10 mL of petroleum ether. After centrifugation, 3 mL of the ethereal supernatant was read at a wavelength of 490 nm using a spectrophotometer against petroleum ether.

The standard used was a carotene solution of concentration 1.12 g/L. The percentage of vitamin A was determined by the following calculation:

%A= CA x Vt /M₀ x 100

With: %A: Percentage of vitamin A; CA: Concentration of vitamin A; Vt: Total volume; M₀: Mass of leaf extract

Vitamin C content

The vitamin C content was determined by the oxidative reaction of ascorbic acid with iodine from the reaction between iodate and iodide in an acid medium (Hadziyev and Steele, 1976). Indeed, 10 g of fresh *Pennisetum purpureum* leaves were mixed with 100 mL of 2% HCl. Then 1 mL of the mixture was combined with 0.5 mL of 1% KI and 2 mL of 0.5% starch solution. The whole mixture was then titrated with 0.001 N KIO₃ solution under stirring until it turned persistent blue.

The vitamin C content was given by the ratio

%C = (Ve-Vb) x N x 88 x Vt / m x V x 100 Protein content in g/100 g

With: %C = Ascorbic acid content in mg in 100 g of fresh material; Ve = volume (mL) of KIO₃ used for titration; Vb: = volume (mL) of KIO₃ used for the blank (control); Vt: total volume of the extract; N: = normality of KIO₃ (0.001 N); 88: = weight of milliequivalent ascorbic acid; m = mass of ground fresh material (g); V = volume of the titrated extract

Vitamin B6 content

The vitamin B6 content was determined by the method used by Strohecker et *al.*(1965) whereby a mass M_0 of *Pennisetum purpureum* leaves was mixed with a 0.1 N HCl solution. Then 1 mL of the mixture was combined with 1 mL of methanol, 0.5 mL of 3% NaOH, 4 mL of distilled water and 4 drops of 2% potassium ferricyanide. The absorbance was measured at 550 nm. Pyridoxine was used as standard. The vitamin B6 content is expressed as a percentage by the ratio: %B= C_{B x} Vt/ M₀ x 100

with: %B: Percentage of vitamin B6; C_B: Concentration of vitamin B6; Vt: total volume, M₀: mass of leaves.

Mineralization

The mineralisation was carried out by the method of Randhawa (2005), according to which 1 g of dried *Pennisetum purpureum* leaf sample was calcined in a muffle furnace for 4 hours until ash was obtained. The ash obtained was mixed with 5 mL of 6 M HNO_3 and heated until 1 mL remained. Then, 5 mL of 3 M HND_3 was added and heated for a few minutes. After filtration, the filtrate was mixed with distilled water to obtain a volume of 50 mL. The solution obtained was called mineralizer and was used for the determination of minerals.

Iron content

The iron content was determined by mixing 2 mL of the mineralisate with 1 mL concentrated H_2SO_4 , 1 mL concentrated H_3PO_4 and 5 mL distilled water. After the addition of 3 drops of 1% diphenylamine, the whole was titrated with a 0.01 N K₂Cr₂O₇ solution until a persistent violet-blue coloration appeared.

Phosphorus content

The phosphorus content was determined by the method of Randhawa (2005), whereby 2 mL of the mineralisate was mixed with 1 mL of molybdic and 1 mL of reducing solution. The absorbance was read at 420 nm. The standard used was a phosphoric solution treated under the same conditions.

Total nitrogen content

The total nitrogen content of the dried *Pennisetum purpureum* leaf sample was determined as a function of the percentage of crude protein, according to the ratio described by Bremner (1965).

Phytochemical study

Treatment of plant material

The plant samples were freeze-dried, powdered, stored at room temperature and protected from light. Each sample (20 g) was mixed with 250 mL of water. The mixture was boiled for 60 min. The extracts were filtered, concentrated, freeze-dried and stored in sterile bottles at 4 $^{\circ}$ C.

Phytochemical screening of the aqueous extracts

Phytochemical screening was carried out to identify the different important chemical groups. Standard phytochemical screening procedures used by Harborne (1998) and Sima et *al.* (2019) were applied

in this study. These tests are based on the appearance of characteristic colourations after the addition of specific reagents.

Quantitative study of phenolic compounds

Determination of total phenols: The total phenol content of the crude extracts or fractions was determined by the method of Folin-Ciocalteu (Vernon et *al.*, 1999). The absorption is read by a spectrophotometer at the wavelength of 735 nm.

Determination of total flavonoids: The total flavonoid content of the crude extracts and fractions was determined by the aluminum trichloride method Sima-Obiang (2018). Absorbances were measured at a 435 nm spectrophotometer.

Determination of the total tannins: Tannin content was determined by using the Standard Method for Determining the Tannins in Sorghum (SMDTS, 1984). Absorbances were measured at 525 nm with a spectrophotometer.

Determination of the total proanthocyanidins: The proanthocyanidin assay is demonstrated by HClbutanol method (Obiang et *al.*, 2021). Optical densities were read at a wavelength of 550 nm at the spectrophotometer.

Pharmacological activities Antioxidant activity index (AAI)

The AAI based on DPPH was estimated by the method of Scherer and Godoy (2009). A concentration range of 0.78 to 100 μ g/mL was prepared for each extract. Ascorbic acid (vitamin C) and BHA were used as controls. The absorbance was measured at 517 nm. The percentage inhibition was obtained by the following formula:

% Radical scavenger activity = [(Absorbance of DPPH - Absorbance of sample)/ Absorbance of DPPH] × 100

The concentration of extracts reducing 50% of DPPH (IC₅₀) was determined from the curve of the percentage inhibition in the function concentration of

the extract. The AAI was calculated using the following formula:

AAI = Final DPPH concentration/IC₅₀

According to the criteria of Scherer and Godoy (2009), plant extracts show low antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI is between 0.5 and 1.0, high activity when AAI is between 1.0 and 2.0, and very high activity when AAI > 2.0

Total antioxidant activity

The total antioxidant activity test is based on the reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Ngoua et *al.*, 2018). A total volume of 0.3 mL of extract dissolved in methanol was added to 3 mL of reagent solution (0.6 mol sulphuric acid/sodium phosphate 28 mmol/L and ammonium molybdate 4 mmol/L).

The mixtures were incubated at 70°C for 90 min, then cooled to room temperature. The absorbance was measured at 695 nm using a spectrophotometer. The total antioxidant activity was expressed as the number of equivalents of ascorbic acid, BHT and quercetin.

Protein denaturation test

The albumin denaturation method was used (Ngoua-Meye-Misso et *al.*, 2018) with slight modifications. 0.1 mL of fresh chicken egg albumin with 1.9 mL of phosphate-buffered saline (PBS, pH 6.4) and 1 mL of variable concentration of the aqueous extract. Incubations were then carried out at 37°C for 20 min, followed by heating at 70°C for 10 min. After cooling, the absorbances were measured at 660 nm with a spectrophotometer. Diclofenac sodium was used as a reference.

Inhibition (%) = [(Sample - Abscontrol) / Abscontrol] x 100.

Abs = absorbance. The concentration of the extract for 50% inhibition (IC₅₀) was determined by the dose-response curve.

Antibacterial activities

The diffusion method was used to study the susceptibility of microorganisms to plant extracts. The antibiotics were used as positive controls.

Bacterial germs tested

The microorganisms used in the study consisted of two reference strains (*Staphylococcus aureus* ATCC25293 BHI and *Escherichia coli* 105182 CIP) and two wild-type strains (*Staphylococcus aureus* and *Salmonella typhi*).

Determination of relative percentage inhibition

The relative percentage inhibition of the test plant extract with respect to positive control was calculated by using the following formula (Obiang et *al.*, 2017). Relative percentage inhibition of the test extract = $100 \times (X-Y) / (Z-Y)$ Where X is the total area of inhibition of the test extract, Y is the total area of inhibition of the solvent, and Z represents the total area of inhibition of the standard drug.

Statistical analysis

The tests were performed in triplicate to ensure reproducibility of the result. The data were divided into qualitative and quantitative variables. The results of the variables were given in percentages (%). Data were expressed as mean ± standard deviation (SD).

Results

Nutritional compounds

The results of the macronutrients of *Pennisetum purpureum* have been recorded in Table 1. The macronutrient analysis shows that the leaves of *Pennisetum purpureum* have a high moisture content (79%) and crude cellulose (25%). The percentage of lipids (12.64%) is higher than protein (5.6%) and ash (8%). The results also show very low levels of total sugars (0.055%) and citric acid (0.002%).

Table 2 summarises the results for some minerals from the leaves of *Pennisetum purpureum*. The study shows that *Pennisetum purpureum* contains 3.35% iron, 7.45% phosphorus and 0.90% nitrogen. The results of the determination of some vitamins (Table 2) contained in the leaves of *Pennisetum purpureum* show that vitamin A (16.33 mg/mL) is higher than vitamin B6 (3.11 mg/mL) and vitamin C (0.05 mg/mL).

Table	1.	Some	macronutrients	of	Pennisetum
purpur	еит				

Macronutrients	% in dry matter		
Fat	14.64		
Total protein	5.60		
Total sugars	0.055		
Citric acid	0.002		
Crude fibre	25		
Ash	8		
Humidity	79		

Phytochemical and pharmacological studies

The results of the phytochemical screening and the determination of phenolic compounds in the leaves of *Pennisetum purpureum* are summarised in Tables 3 and 4, respectively. The qualitative study shows that saponosides, total phenolic, coumarins and triterpenoids are very abundant. Other secondary metabolites such as tannin gallic, total flavonoids, alkaloids and anthracenosids are abundant in *Pennisetum purpureum* extracts.

Table 2. Dosage of some vitamins and minerals fromPennisetum purpureum.

Vitamins	Vitamin C	0.05 mg/mL		
	Vitamin A	16.33 mg/mL		
	Vitamin B6	3.11 mg/mL		
Minerals	Iron (Fe)	3.35%		
	Phosphorus (P)	7.45%		
	Nitrogen (N)	0.90%		

The quantitative tests for secondary metabolites show that *Pennisetum purpureum* extract has 1331.83 \pm 33.30 mg (GAE)/100 g of phenolic compounds, 52.90 \pm 5.14 mg (QE)/100 g of total flavonoids, 176.00 \pm 6.15 mg (EPA)/100 g of proanthocyanidin and 46.88 \pm 3.55 mg (TAE)/100 g of total tannins.

The results of the antioxidant activity (Table 4) by DPPH radical scavenging show that the *Pennisetum* purpureum extract (IC₅₀ = 29.28 ± 0.70 µg/mL; AAI = 1.60) has a lower activity than the standards, in particular vitamin C (IC₅₀ = 7.12 ± 0.60 µg/mL; AAI = 7.02) and butylated hydroxyanisole (IC₅₀ = 6.59 ± 0.30 µg/mL; AAI = 7.58 µg/mL). The determination of total antioxidant activities by the Phosphomolybdenum assay shows that *Pennisetum purpureum* leaves possess 8.99 ± 0.65 mg/g quercetin equivalent extract and 52.95 ± 3.56 mg/g vitamin C equivalent extract.

Table 3. Phytochemical screening of *Pennisetum purpureum*.

Chemical groups	Aqueous extract
Saponosids	+++
Tannin gallic	++
Tannin catechic	+
Total phenolic	+++
Total flavonoids	++
Reducing sugars	+
Alkaloids	++
Proanthocyanidins	+
Anthracenosids	++
Coumarins	+++
Triterpenoids	+++

+++ = Very abundant; ++ = Abundant; + = Very little; - = Absent.

The inhibition of protein denaturation by *Pennisetum* purpureum extracts (Table 4) shows that the extracts studied (IC₅₀ = $65.05 \pm 3.43 \ \mu g/mL$) showed a lower

inhibition of ovalbumin denaturation compared to the standars (IC₅₀ = $35.75 \pm 2.37 \ \mu g/mL$).

Tetracycline was used to determine the relative percentage inhibition of *Pennisetum purpureum* antibacterial activity (Fig. 1). The extracts showed maximum relative percentage inhibition against *Staphylococcus aureus* ATCC25293 BHI (100%), *Escherichia coli* 105182 CIP (111.64%), *Staphylococcus aureus* (112.5%), *Salmonella typhi* (81.25%).

Discussion

The nutritional study of Pennisetum purpureum leaves shows the presence of several macromolecules with high moisture and crude cellulose content. This result justifies why guinea pigs consume this food without additional hydration. The presence of other nutrients (lipid, protein, ash, total sugar and citric acid) confirms the diversity and richness of Pennisetum purpureum. This is why these leaves are easily consumed by ruminants (sheep, goats, cows,..) as fodder. Our results are in agreement with the work of Bedru (2019). Mineral analysis showed that Pennisetum purpureum contains phosphorus in the majority and nitrogen in very small quantities. These levels of phosphorus and iron are essential for the formation of bones and teeth (Saghir et al., 2022) and to reduce infections and anaemia in ruminants (Trombetti et al., 2022).

Table 4. Dosage of phenolic compounds, antioxidant activity and inhibition of protein denaturation by aqueous extracts of *Pennisetum purpureum*.

Phenolic compounds	Total phenols			1331.83 ± 33.30 mg (GAE)/100 g
	Tot	52.90 ± 5.14 mg (QE)/100 g		
	Proa	176.00 ± 6.15 mg (EPA)/100 g		
	To	46.88 ± 3.55 mg (TAE)/100 g		
Antioxidant activity	DPPH test	Extract	IC ₅₀	$29.28 \pm 0.70 \ \mu g/mL$
			AAI	1.60
		Vitamine C	IC ₅₀	$7.12\pm0.60~\mu\text{g/mL}$
			AAI	7.02
		BHA	IC ₅₀	$6.59 \pm 0.30 \ \mu g/mL$
			AAI	7.58 μg/mL
	Phosphomolybdenum test	VtCE		52.95 ± 3.56 mg/g
	QE			$8.99 \pm 0.65 \text{ mg/g}$
Inhibition de la dénaturation	Test de dénaturation	Extrait	IC ₅₀	$65.05 \pm 3.43 \mu g/mL$
des protéines		Diclofenac de sodium	IC ₅₀	35.75 ± 2.37 μg/mL

GAE=Gallic acid equivalent, QE=Quercetin equivalent, ATE=Acid tannic equivalent, APE=Apple procyanidins equivalent, VtCE=Vitamine C equivalent.

The results of the determination of some vitamins show that in *Pennisetum purpureum* extract, vitamin A is in the majority, vitamin B6 in reasonable amounts and vitamin C in very small amounts. This high level of vitamin A is essential for good nutrition in ruminants to ensure growth. This reasonable concentration of vitamin B6 is important for the regulation of amino acids and hormones in the body (Li et *al.*, 2021). All of the above assays show a very interesting nutritional profile of *Pennisetum purpureum* leaves. Indeed, the leaves of this plant are a good food source, but apart from this interesting nutritional composition, the pharmacological side is not left out.



Fig. 1. Determination of relative percentage inhibition of water extracts from Pennisetum purpureu.

The phytochemical screening of the extract of the leaves of Pennisetum purpureum shows that this plant has a majority of saponosides, total phenolic, coumarins and triterpenoids. It has abundant tannin gallic, total flavonoids, alkaloids and anthracenosids. These results are in agreement with Obiang et al. (2021), who presented a similar profile in his work on the stem extract of this plant. The presence of secondary metabolites may confer several pharmacological activities to this plant, such as antioxidant, anti-carcinogenic, anti-angiogenic, antiinflammatory, antibacterial, antifungal, anti-anemic effects (Misso et al., 2020). Several researchers have shown that polyphenols are highly active against inflammatory diseases and infections of bacterial origin (Obiang et al., 2021). Indeed, the presence of antioxidant, anti-inflammatory and antibacterial effects has been confirmed in the leaves of this plant. Despite the low antioxidant and anti-inflammatory activities, this plant shows the highest relative inhibition percentages against several bacteria. The

leaves of *Pennisetum purpureum* used by guinea pig breeders are rich in nutritional and therapeutic compounds.

Conclusion

Pennisetum purpureum (family Poaceae) is cultivated in tropical and subtropical regions. It was found that the leaves not only have a nutritional contribution (carbohydrate, lipid, starch, cellulose, protein, acidity, iron, nitrogen, phosphorus, vitamins A, B6 and C) but also a moderate therapeutic contribution. Thus, the leaves of *Pennisetum purpureum* have a nutritional and therapeutic profile that meets the needs of ruminants in livestock farming.

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Competing interests

The authors declare that they have no competing interests.

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