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Phytochemical Compositions and *In vitro* Antioxidant Capacity of Methanolic Leaf Extract of *Axonopus Compressus* (P. Beauv.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author BOI conceptualized and designed the work, interpretation of results, laboratory analysis and drafting of the original manuscripts and final approval of the version. Author EM involved in the project design, involved in result interpretation and laboratory analysis. Critical revision of draft article for suitability and intellectual content and final approval of the version. Author HJB involved in statistical analysis and critical revision of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: We evaluated the phytochemical contents and antioxidant capacity of the methanolic leaf extract of the Nigerian *Axonopus compressus.* This is a preliminary investigation to determining the active principle which may be involved in the antidiabetic mechanism of the plant.
Study Design: Phytochemicals and antioxidant capacity were determined using chromatographic and spectrophotometric detection methods of cold leaf extracts of *Axonopus compressus*.
Place and Duration of Study: Department of Biochemistry, College of Natural and Applied Sciences, Michael Okpara University of Agriculture Umudike Abia State, Nigeria.
Methodology: Antioxidant activities were investigated by three tests namely: 2,2-

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diphenyl-1-picryl hydrazyl (DPPH), Fe^{3+} to Fe^{2+} transformation (ferric reducing antioxidant power, FRAP) and a modified version of TBARS assay. These *in vitro* antioxidant models were carried out after cold extraction maceration. The antioxidant capacity was measured at varying concentrations (10 ~ 400 µ/ml) of the extract required to quench the free radicals by 50% (IC_{50}) and expressed as % inhibition. Phytochemicals were determined by standard detection and spectrophotometric methods.

Results: The phytochemicals: saponin (1.2±0.1), alkaloid (2.10±0.12), tannin (0.71±0.4), flavonoid (1.92±0.13) and polyphenol (1.78±0.21) in mg/100g were strongly detected. The leaf extract was found to have a concentration dependent antioxidant activity comparable with that of ascorbic acid. *Axonopus compressus'*s DPPH reduction was highest at 400µg/ml (92.00 ± 0.002%) with IC_{50} of 52.2µg/ml. The ferric reducing power of the extract at 400µg/ml (78±1.83% [FRAP:0.92]) and the inhibition of lipid peroxidation measured as TBARS was 92.±1.21%

Conclusion: The presence of these phytochemicals and the high antioxidant power may explain the astringent action of the plant observed in its ethnomedicinal use especially in the treatment of diabetes. Our findings therefore, suggest that *Axonopus compressus* possess a strong antioxidant property that may substantiate its ethnomedicinal efficacy.

Keywords: Antioxidants; phytochemicals; flavonoid; medicinal plant; Axonopus compressus; Nigeria.

1. INTRODUCTION

Medicinal plants are significant source of synthetic and herbal medications. In most rural and urban areas of sub-Saharan Africa such as in Southern-Nigeria, medicinal herbs are used as raw drugs, extracts and/or tinctures [1]. The past few decades have witnessed rapid progress in the use of plant phytochemicals and herbal products as popular and alternative treatment remedies [2-4]. More recent studies have reported its use in phytoremediation of hydrocarbon-contaminated soil [5]. Specifically, *Axonopus compressus* a perennial, terrestrial, stem compressed grass with a bearded or hairy nodes belonging to the family Poaceae [6] commonly known as carpet grass (with the symbol AXCO) is widely used in the Southern part of Nigeria to treat diabetes mellitus [7]. This herb however, is believed to have no toxicity [8,9]. Our group has recently reported the antidiabetic activity of the methanolic leaf extract of *Axonopus compressus* (P.Beauv) in alloxan-induced diabetic rats [10]. Therefore, the present study was undertaken to evaluate the phytochemical contents and antioxidant capacity of the Nigerian *Axonopus compressus leaf* extract. This is a preliminary investigation to determining the active principle that may be involved in the antidiabetic mechanism of the plant hence its effectiveness in ethnomedication.

It is known that phytochemicals generally refer to chemicals that may affect healthy status but are not yet established as essential nutrients [11,12]. Some of the known phytochemical groups already identified in plants include: anthocyanin, caroteinoids, flavonoids and tannins [13-15]; alkaloids, saponins, monophenols and phenolic acids [16-18]. These phytochemicals are a rich source of antioxidants to the plants [19]. Several studies suggest that plants rich in antioxidants play a protective role in health and against diseases [20-21]. Current research works have also shown that these phytochemicals can protect against human diseases through their antioxidant activity [22-23].

Antioxidants however are molecules capable of inhibiting the oxidation of other molecules. Oxidation, a chemical vector that transfers electrons from a substance to an oxidizing agent

[24] produces free radicals which in turn starts chain reaction that damages cell [25]. This oxidation basically, has been implicated as one of the mechanisms of action of diabetes disease [26]. Antioxidants on the other hand interfere with the chain reaction by removing free radical intermediates and inhibit other oxidation reactions. Generally, the relative interaction between the different antioxidants is a complex one with the various metabolites and enzymes having synergistic and interdependent effects on one another [27-28]. Therefore, the action of one antioxidant may depend on the proper functioning of other members of the antioxidant system.

Obviously, it is becoming evident that medicinal plants have a potential in today's synthetic era as cases of drug resistance increases. Some studies have estimated that only 20% of the plant flora has been studied and 60% of synthetic medicines owe their origin to plants. Scientific reports on the antidiabetic efficacies and mode of action of *Axonopus compressus* seems scanty. It is generally observed that interest in *Axonopus compressus* have concentrated more on screening for hypoglyceamic action rather than probe into its antidiabetic/hypoglycaemic mechanisms of action. The diverse composition and activity of chemical/biological species in this plant may likely place it at advantage position over orthodox chemotherapeutic agents in the management of complex diseases such as diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Matured fresh leaves of *Axonopus compressus* were collected from natural habitat in Micheal Okpara University of Agriculture Umudike, Nigeria (Latitude 05º 29^1 N to 05º 42^1, Longitude 07º 24^1 E to 07º 33^1) in the month of June 2010 and identified by Dr. Dike in the Forestry Department, College of Natural Resources and Environmental Management, Micheal Okpara University of Agriculture Umudike, Nigeria. A voucher specimen with the number Ibeh 2010-56 was deposited in the University herbarium for future reference.

2.2 Preparation of Plant Extract

The leaves were washed with distilled water without squeezing to remove debris and dust particles, air-dried at room temperature and pulverized into a uniform material using a Thomas-Willey mini-milling machine (model 4, 3375-e25). Extraction was done by cold maceration in 80% methanol for 48h with intermittent shaking every 2h. The extract was then filtered with Whatman filter papers no. 42 (125mm) and the filtrate was evaporated to dryness in an electric oven at 40ºC. The obtained crude extract was packed in air-tight plastic containers and stored in a refrigerator at 4ºC until time of use. The percentage yield of the extract was calculated using the formula below:

$$\% \text{ Yield} = \frac{\text{weight of the extract}}{\text{weight of plant material}} \times \frac{100}{1}$$

2.3 Phytochemical Screening and Quantification

The detection of major chemical groups was carried out by thin-layer chromatography (TLC) on silica gel 60 F_{254}, layer thickness 0.25mm (Merck, Darmstadt, Germany) after dissolving 2mg of the extract in 2ml of methanol. The plates were developed, then left to dry for about

10 min before viewing under UV fluorescence light at 254 and 366nm. Finally, spraying was done with the required detection reagent (Dragendoff, Ferrocynide and Vanillin) to determine the compounds present and the solvent system which gave the best observation. For flavonoids, TLC was developed in n-butanol/acetic acid/water (4:1:5), then spots were visualized with 1% $AlCl_3$ solution in methanol under UV light (366nm) (Ce 3041 Buck Scientific, UK). The methods of Harborne [29,30] and Trease and Evans[31] were used to identify the following phytochemicals in the extracts; alkaloids, saponins, tannins, anthraquinones, flavonoids, terpenoids, steroids and cardiac glycosides. Quantitative analysis of the phytochemicals was determined by methods variously described by Trease and Evans [32], Sofowara [33] and Harborne [34].

2.4 Total Flavonoid Content (TFC)

Total flavonoid content was determined by the aluminum colorimetric method [35] using Quercetin as a standard.

2.5 Antioxidant Assay

2.5.1 Determination of DPPH radical scavenging activity

Here rapid thin layer chromatography (TLC) screening for antioxidant activity was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. The plates were developed in methanol: ethyl acetate (2:1) and afterwards air-dried and sprayed with 0.2% w/v DPPH spray in methanol. This was visualized for the presence of yellow spots. Radical scavenging activity of extracts was performed according to the DPPH spectrophotometric method of Mensor et al.[36] using vitamin C (Emzor Pharmaceutical Industries, Nigeria) as a positive antioxidant control. Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank while 1 ml of 0.3 mm DPPH plus methanol (2.5 ml) was used as a negative control.The free radical scavenging properties of the extracts against 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical were measured at 518 nm as an index to their antioxidant activity. In its radical form, DPPH absorbs at 518 nm but upon reduction by an antioxidant or a radical species, the absorption decreases. The concentrations of the extracts and vitamin C used were 10, 50, 100, 200 and 400 µg ml^{-1}. The absorbance (abs) of the resulting mixture measured at 518 nm were converted to percentage antioxidant activity (AA %). Also the free radical scavenging activity was obtained as the percentage DPPH decolourization of the sample and thus calculated by the equation:

AA% = [100 – ((ABS sample —ABSblank) × 100)] /ABS control

The assay was carried out in triplicates for each concentration. The IC_{50} values obtained shows the concentration of extracts required to scavenge 50% of DPPH free radicals.

2.5.2 Ferric reducing antioxidant power (FRAP) assay

The reductive potential of *Axonopus compressus* was determined according to the method of Benzie and Strain [37] based on the chemical reduction of Fe^{3+} to Fe^{2+}. At low pH, reduction of ferric tri(2-pyridyl)-1,3,5-triazine (Fe III TPTZ) complex to ferrous form (an intense blue colour) can be monitored by measuring the change in absorption at 593nm. The change in absorbance is therefore directly related to the combined or total reducing power of

the electron donating antioxidant present in the reaction mixture. The calculation was done by:

FRAP value of sample (µM)=

$$\frac{(\Delta \text{ in absorbance of sample from 0−4 min}) \times \text{FRAP value of standard (1000µm)}}{(\Delta \text{ in absorbance of standard from 0 to 4 min})}$$

2.5.3. Inhibition of lipid peroxidation

A modification of thiobarbituric acid reactive substances (TBARS) assay was used to determine the level of lipid peroxide formed using egg yolk homogenate as lipid-rich media [38]. Egg homogenate (0.5 ml, 10% v/v) was added to 0.1 ml of extract (1mg/ml) and the volume made up to 1 ml with distilled water. Then, 0.05 ml of $FeSO_4$ was added and the mixture incubated for 30 minutes. Acetic acid (1.5 ml) and thiobarbituric acid (1.5 ml) in SDS was sequentially added. The resulting mixture was vortexed and heated at 95ºC for 60 minutes. After cooling, 5 ml of butanol was added and the mixture centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532 nm and converted to percentage inhibition using the formula:

Inhibition of lipid peroxidation (%) = (1 – E/C) x 100

Where C = absorbance of fully oxidized control and E = absorbance in the presence of extract

2.6 Statistical Analysis

Results were presented as mean ± standard error of mean (SEM) and the statistical analysis was done using one way analysis of variance (ANOVA), SPSS version 17. The differences between the means were tested using Post Hoc LSD. A *p*-value of $P<0.05$ was considered to be statistically significant. All antioxidant assays were done in triplicates.

3. RESULTS

3.1 Plant Extraction

The yield of the methanolic leaf extract of *A. compressus* was 4.87% w/w of the dry matter and was greenish in colour.

3.2 Phytochemical Content

The results of the preliminary phytochemical screening of *Axonopus compressus* revealed the presence of steroids (steroid glycoside), alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phlobatannins, anthraquinones and terpenes (Table 1). However the quantitative analysis yielded high levels of flavonoids (1.92±0.13), alkaloids (2.10±0.12), polyphenols (1.78±0.21) and moderate levels of tannins (0.71±0.40) and saponins (1.2±0.10) (Table 2).

3.3 Antioxidant Activity *In vitro* Analysis

The *in vitro* percentage inhibition of DPPH by *Axonopus compressus* and vitamin C, ferric reducing antioxidant power and the inhibition of lipid peroxidation (measured as TBARS) of the extract revealed a concentration-dependent antiradical activity (Table 3, Fig. 1). In the case of DPPH, the extract generally had an insignificantly higher DPPH reduction capacity at 200 µg/ml (88±0.0.01) and 400 µg/ml (92±0.002) concentrations when compared with the scavenging activity of vitamin C, a known antioxidant used as positive control. IC_{50} values for *Axonopus compressus* and ascorbic acid were 52.20 and 56.10 µg/ml, respectively (Table 3). The ferric reducing power of the extract at 400 µg/ml was 78±1.83% (FRAP value=0.92) and that of inhibition of lipid peroxidation (measured as TBARS) was 92.±1.21% (Fig. 1).

The reducing power of vitamin C and *Axonopus compressus* increased gradually with increasing concentration of the extract.

Table 1. Phytochemical screening of leaf extracts of *Axonopus compressus*

Plant Metabolite	Extract Content
Cardiac glycosides	+++
Steroid glycosides	++
Saponins	+ +
Tannins	++
Alkaloids	+++
Phlobatannins	+
Terpenoids	++
Flavonoids	+++
Anthraquinones	++

+ = Trace, ++=Moderate, +++ = Abundant

Table 2. Phytochemical composition of the leave extract of *Axonopus compressus* expressed as mg/100 g dry weight

Plant Metabolite	Composition
Polyphenols	1.78±0.21
Saponims	1.20±0.10
Tanins	0.71±0.40
Alkaloid	2.10±0.12
Flavonoids	1.92±0.13

Results are mean of triplicate determinations on a dry weight basis ± standard deviation

Table 3. Antioxidant activity measured as % Reduction of DPPH

Concentration(µg/ml)	% Antioxidant Activity (% Inhibition)	
	Axonopus compressus	**Ascorbic Acid**
10	40.93±0.020	72.00± 0.060
50	48.64±0.007	76.00±0.080
100	72.00±0.011 *	77.00±0.040
200	88.00±0.001*	81.00±0.002
400	92.00±0.002*	87.00±0.110
	52.2ǂ	56.1ǂ

**indicates no significant difference at (P>0.05); ǂindicates IC50 value measured at µg/mL*

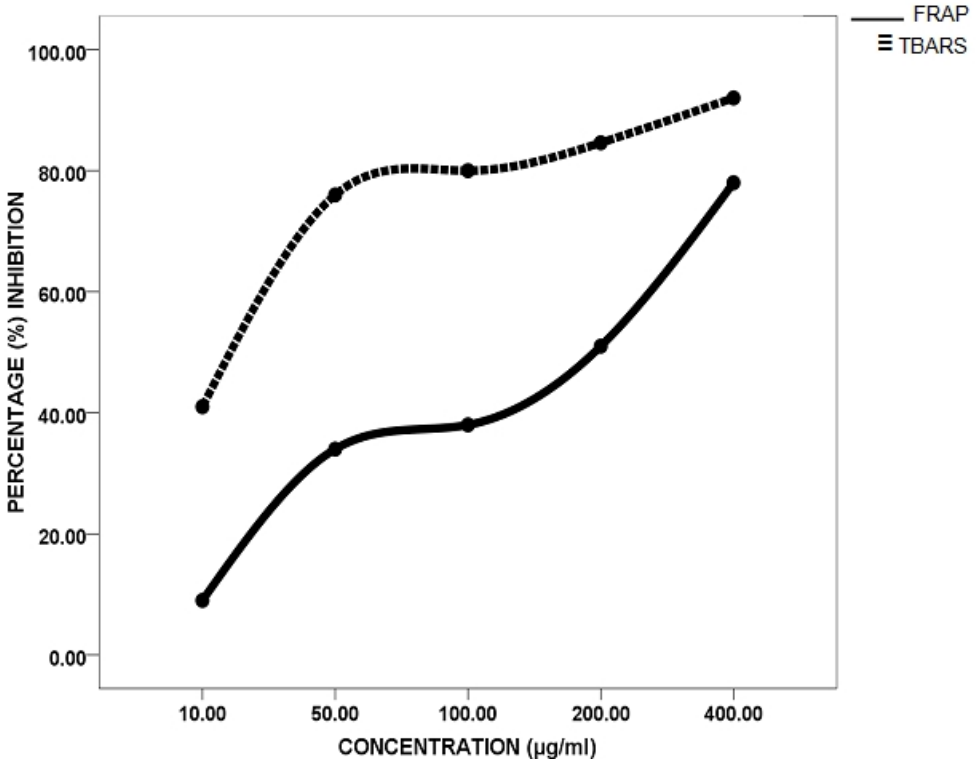

Fig. 1. Ferric reducing potential (FRAP) and inhibition of lipid peroxidation (TBARS) by *Axonopus compressus (P.Beauv.).*

4. DISCUSSION

Our study summarily tend to reveal that secondary metabolites such as alkaloids, tannins, flavonoids and cardiac glycosides present in *Axonopus compressus* may be connected to its high antioxidant activity which may be related to curative and/or management potential of many ailments claimed in its ethno-medicine most especially diabetes.

Qualitative analysis showed strong presence of cardiac glycosides, alkaloids, and flavonoids others also present include steroid glycoside, saponin, tannin, terpenoids, anthraquinone and trace quantity of phlobatannins (Table 1). This agrees with the work of Ogie-Odia et al. [39] on qualitative detection of phytochemicals of *Axonopus compressus.*

Quantitative measurement showed that the leaf sample have a high content of flavonoid, alkaloid and polyphenol with moderate levels of tannin and saponin. This high content of flavonoid and alkaloid may play a role in the plants therapeutic effectiveness. It is generally known that these compounds (flavonoids and alkaloids) can inhibit alpha-glucosidase activity to depress the glucose level in blood [40]. It has been demonstrated that alkaloids and flavonoids could inhibit alpha-glucosidase activity cooperatively which, would successfully depress blood glucose levels in antidiabetic therapy. Some researchers have evaluated the chemical structures of flavonoids responsible for its inhibitory activity especially in Yeast. Also anthocyanidin, isoflavone, and flavonol groups with IC_{50} values less than 15µM has been shown to inhibit Yeast and rat α-glucosidase [41]. Several works have confirmed that

isolates of alkaloids named piperumbellactam A (10), piperumbellactam B (11) and piperumbellactam C (12) from branches of *Piper umbellatum* have moderate α-glucosidase enzyme inhibition with IC_{50} values 98.07 ± 0.44, 43.80 ± 0.56, and 29.64 ± 0.46, respectively [42,43]. Pfundstein et al. [44] equally showed that some phenol methanolic isolates from dried *Terminalia chebula* (Combretaceae) fruits have antidiabetic activity. Furthermore, tannin has been shown to have antidiabetic effect in human T2D patients and also to induce glucose transport through activation of the insulin-mediated signaling pathway in adipocytes [45]. Similarly, saponin have both hypoglycemic and alpha glucosidase inhibitory effects [46]. Our data (Table 2) recorded a significant level of saponin (1.2±0.10) component which may likely contribute to the plants mechanism of action on reducing diabetes (hypoglycemic activity).

We also evaluated the antioxidant properties of the extracted components using different antioxidant assays. The antioxidant attributes of *Axonopus compressus* as affected by alkaline hydrolysis and the release of bound phenolics have limited experimental evidence. It is of note that the antioxidant capacity of phenolic compounds is mainly due to their redox properties which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [47]. Our results showed a high antioxidant capacity (inhibition of DPPH [Table 3], FRAP [Fig 1] and TBARS [Fig 1]) of the extract thus data presented here showed that the antioxidant activity were concentration dependent having maximal effect at 400µg/ml. The DPPH activity obtained indicates that our extract may have a comparable antioxidant capacity with that of ascorbic acid requiring 52.2 µg/ml (IC_{50} value) to reach 50% inhibition of DPPH radical activity, a value lower than ascorbic acid (56.1µg/ml). A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. It also has a significantly (92±0.002) higher scavenging effect on the DPPH radical activity at 400µg/ml concentration when compared with ascorbic acid (87±0.11). This may suggest a better antioxidant capacity of the extract. DPPH however is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [48]. DPPH radical generally is regarded to be a model for lipophilic radical activity. Positive DPPH test suggests that the samples were free radical scavengers. The ferric reducing power of the extract at 400 µg/ml/ml gave 78±1.83% (FRAP value=0.92) and that of inhibition of lipid peroxidation (measured as TBARS) was 92.±1.21%.The inhibition of TBARS a measure of the oxidative stress was high suggesting that *Axonopus compressus* is a good antioxidant source. This concurs with previous studies as reported by Trease and Evans [32] that secondary metabolites such as alkaloids, tannins, flavonoids and cardiac glycosides present in the plant are the basis for the curative and or management of many ailments such as wounds, digestive disorders, coughs, ulcers, skin troubles and different kinds of inflammations claimed in its ethno-medicine. Generally, the antioxidant reaction of *Axonopus compressus* is concentration-dependent which means that an increase in antioxidant activity is linearly dependent on the methanolic leaf extract concentration of the plant (Fig. 1 and Table 3). All extracts at tested doses (100-400 µg mL^{-1}) revealed good scavenging activity for DPPH, FRAP and inhibition of TBARS in a dose-dependent manner. We observed a slightly higher activity of the extract on DPPH when compared with ascorbic acid, a standard antioxidant agent (IC_{50} = 52.2 Vs 56.1 µg mL^{-1}) (Table 3). Thus hydroxyl radical scavenging capacity of the extract is directly related to its antioxidant activity. The effect of methanolic leaf extract of *Axonopus compressus* on the inhibition of free radical-mediated lipid peroxidation (here measured as TBARS) and reduction of ferric tripyridyl traizaine (Fe III TPTZ) complex to ferrous form at 593nm is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture assessed. The ability of the above mentioned extracts to inhibit TBARS and reduce Fe^{3} to Fe^{2} seems to be directly related to the prevention of propagation of the process of lipid

peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of the chain reaction.

Furthermore, flavonoids and other phytochemicals have been demonstrated to generally have antioxidant effects. Flavonoids are one of the most numerous and widely spread groups of phenolic compounds in higher plants. Some of them due to their phenolic structure are known to be involved in the healing process of free radical mediated diseases including diabetes [49]. Therefore, the phytochemicals present in *Axonopus compressus* contributes to its antioxidant property, since diabetes may be mediated through free radicals. The extract thus possesses compounds that may serve as the anti-diabetic principle agent.

4. CONCLUSION

Our results showed that *Axonopus compressus* is rich in phenolic constituents and demonstrated good antioxidant activity, measured by TBARS, FRAP and DPPH assay models. Moreso, the chromatographic separation enabled the identification of a wide range of phenolic compounds present in this plant without time consuming sample preparation or previous fractionation. Further studies are necessary to characterise the identified compounds and seek for novel phenolic species and the consequent test of their antidiabetic activity. *Axonopus compressus* could be a good source of natural antioxidants. Future studies are necessary to determine *in vivo* activity and bioavailability of the extracts so as to confirm the effectiveness of its ethno-medicinal / beneficial use.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and Michael Okpara University, Umudike, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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