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Elemental Composition, Evaluation of Anti-nutrients, and Antioxidant Potentials of *Morinda lucida*

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOT designed the research work, materials and Protocol and also wrote the first draft of the manuscript for this research work. Authors FAO and TAO designed the statistical analysis and proof read the final dratted manuscript. Author OAT managed the analyses of the study and the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Morinda lucida is a tropical West African tree of medium-size, having brown colored bark, white flowers and yellowish ellipsoid seeds. It is widely used in traditional medicine systems of different countries. The preliminary phytochemical screening of different extracts prepared from bark, leaves and seeds of *M. lucida* revealed the presence of Saponin, Tannins, Alkaloids, Flavonoids, and Cardiac glucosides. Furthermore, it also reveals presence of Minerals like Sodium, Potassium, Calcium, Iron, Zinc, Copper and Phosphorus were confirmed. Anti-nutrient were also present in appreciable percentage quantities. The anti-nutrients: Phytates, Oxalates and Saponins were

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present in different concentrations, Furthermore, the percentage of proximate content and *in vitro* antioxidant potential were also estimated in this current study. The purpose of this present study was to conduct elemental composition, evaluation of anti-nutrient and antioxidant properties of *M. lucida*.

Keywords: Morinda lucida benth (Rubiaceae); qauntitative methods of analysis; mineral composition; anti-nutrients; proximate method; in-vitro-antioxidant analysis.

1. INTRODUCTION

Morinda is a genus of flowering plants in the madder family, Rubiaceae [1]. *Morinda lucida* (Rubiaceae) is a tropical West Africa rainforest tree also called Brimstone tree. In Coted'Ivoire, it is locally called Sangogo or Bondoukou alongua while in Ghana, it is known as Twi, Kon kroma or Ewe amake. Among the Togolese, Ewe amaka or Atak ake, South–Western Nigeria, it is called Oruwo [2,3].

Oruwo is a medium sized tree at maturity. Its stem, bark infusion is used as antimalarial and antidiabetic agent [4]. It showed significant Antimalarial activity [5-9], anti-*Salmonella typhi* activity [5], *M. lucida* extracts induced contractility effect on isolated uterine smooth muscle of pregnant and non-pregnant mice [10,11]. In addition, earlier researchers also evaluates the toxicity and mutagenicity of *M. lucida* extracts [12-14].

The major constituents of *M. lucida* extracts were found to be: essential oils, anthraquinones and anthraquinols [1]. Oruwalol, oruwal, ursolic acid, and oleanolic acid were also isolated from this plant [15,1,5]. The red colorants of *M. lucida* were confirmed to be 1- methyl-ether-alizarin, rubiadin and derivatives, lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin. anthraquinones, oruwacin, tannins, flavonoids, digitolutein, and saponosides were isolated from different parts of *M. lucida*

The root of *M. lucida* is used as chewing sticks are for oral hygiene in Nigeria. The aqueous extracts of different parts of this plant were found to be effective against *Staphylococcus aurens*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* [16-18]. Keeping in view the popular use of different parts of *M. lucida* and evidence based confirmation of different folklore medicinal claims, further investigation was considered mandatory. In the present study chemical constituent and antioxidant potential of different extracts of *M. lucida* were evaluated and the results are presented in the current communication.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Sample

The root, leaf and stem bark from *M. lucida* plants were collected from the tropical rain forest Oshogbo Osun State (Nigeria), in the morning time of 21st of October, 2015. The plant specimen were identified and authenticated at Department of Plant Science and Biotechnology, Adekunle Ajasin University Akungba Akoko, Nigeria where the voucher specimens were kept on record. Vocher number AAU-2418 was recorded for the plant extract for future reference.

2.2 Quantitative Method of Analysis

2.2.1 Saponins

The grinded plant samples (20 g) were extracted with 20% aqueous ethanol by using a water bath maintained at 55℃, for 4 hour with stirring. After filtration the residue was re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml volume separately (water bath temperature was 90℃). Diethyl ether (20 ml) was used for extraction. The process was repeated three times. The ether layer was removed and 60 ml of n-butanol was added to the water layer. Butanol extract was washed with 5% NaCl aqueous solution. After evaporation, the samples were dried in oven to a constant weight; the saponin content was calculated as percentage of the starting material. [19-21,5].

2.2.2 Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath. The dried extracts were weighed and

the test procedure defined by Mahato and Sen 1997 was followed [22,23].

2.2.3 Cardiac glucosides

About 0.5 g of the extract was added to 2 ml of acetic anhydrate plus H_2SO_4. Legal test and the Killer-Kiliani tests were adopted [24,22,25].

2.2.4 Tannins

About 500 mg of the plant sample were weighed and extracted with 50 ml water. Thus obtained filtrate was transferred to a 5 ml test tube containing 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCL and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes and tannin contents were obtained by using standard curve technique [26].

2.2.5 Alkaloids

The plant material (5 g) was weighed into a 250 ml beaker and 200 ml of ethanolic Acetic acid (10%) was added to it. The mixtures were covered and allowed to stand for 4 hour. This was filtered and concentrated on a water bath to one-quarter of the original volume. Ammonium hydroxide (conc.) was added drop-wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered. Thus obtained alkaloidal residue was dried and weighed to a constant mass [26,27].

2.3 Total Phenol (Spectrophotometric Methods)

To 2 g of each sample, 1 ml of diethyl ether was added for defatting. The fat free samples were boiled with 50 ml of ether for 15 min to obtain the phenolic components which were measured at 505 nm following the standard method [17].

3. DETERMINATION OF PROXIMATE ANALYSIS OF *Morinda lucida*

The proximate parameters were determined by using AOAC, 2003 protocol [28]. However, for mineral composition the method developed by Hussain et al. [9] was used [29]. Moisture content [28], Nitrogen estimation [29], Crude proteins [29], Carbohydrates [28], Crude fats [29], Ash values [29], and Crude fibers [30] were estimated by using the standard methodology.

3.1 Elemental Analysis of *Morinda lucida*

The major elements comprising calcium, sodium, potassium and trace elements (Fe and Zn) were determined according to the standard method with slight modification [31,20]. The ground samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were subjected to dry ashing in porcelain crucible at 550℃ in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3 /H_2O_2 (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman filter paper and the volume was made to mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). Concentration of each element was calculated on percentage of dry matter [27].

3.2 Determination of *in vitro*-Antioxidant Conpoments of *Morinda lucida*

3.2.1 Determination of total phenol

The total phenol content of the extract determine by the method of [32]. 0.2 ml of the extract was mix with 2.5 ml of 10% Folinciocalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45^{o}C for 40 mins, and the absorbance was measure at 700 nm in the spectrophotometer, garlic acid would be used as standard phenol. [7,21].

3.2.2 Determination of total flavonoid

The total flavonoid content of the extract was determined using a colourimeteric assay developed earlier [33,34]. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

3.2.3 Determination of ferric reducing property

The reducing property of the extract will be determined by using the earlier reported method where absorbance used was 700 nm [35].

3.2.4 Determination of free radical scavenging ability

The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl)

was determined by using standard procedure [36]. The extract (1 ml) was mixed with 1 ml of 0.4 mM methanolic solution of DPPH the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

3.2.5 Determination Fe2+ chelation

The ability of the extract to chelate Fe2+ was determined using a modified method [8,36]. About 150 mM FeSO4 were added to a reaction mixture containing 168 ml of 0.1 M Tris-HCl, pH 7.4, 218 ml saline and extract and the volume was made up 1ml with distilled water. The reaction mixture will be incubated for 5 min, before the addition of 13 ml of 1, 10-phenantroline and the absorbance will be read at 510 nm.

3.2.6 ABTS scavenging ability

2,2'-Azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability. The ABTS scavenging ability of the extract was determined according to the method describe by [37,38]. The ABTS was generated by reacting 7 mM ABTS aqueous solution with K2S2O8 (2.45 mM/l, final conc.) in the dark for 16 hours and adjusting the absorbance at 734 nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0 ml of ABTS solution and the absorbance was read at 732 nm after 15 min. The TROLOX equivalent antioxidant capacity was subsequently calculated (264.32 g).

3.2.7 Superoxide anion scavenging activity assay

The superoxide anion radicals are produced in 2 ml of phosphate buffer (100 mM, pH 7.4) with 78 µM β- nicotinamide adenine dinucleotide (NADH), 50 µM nitro blue tetrazoliumchloride (NBT) and test samples at different concentrations. The reaction mixture is kept for incubation at room temperature for 5 min. It is then added with 5-methylphenazinium methosulphate (PMS) (10 µM) to initiate the reaction and incubated for 5 min at room temperature. The colour reaction between superoxide anion radical and NBT is read at 560 nm. Gallic acid is used as a positive control agent for comparative analysis. The reaction mixture without test sample is used as control and without PMS is used as blank [32,39].

4. RESULTS

The results of the present study are depicted in Tables 1-5 and Figs. 1-2.

Table 1 shows the Quantitative chemical analysis of minerals present in the *M. lucida* extracts The crude bark extracts of *M. lucida* contains: Potassium (K+), and Sodium (Na), Calcium (Ca2+), Magnesium (Mg2-), Zinc (Zn2-), Iron (Fe2+), Manganese (Mn) and Phosphorous (P+). Lead and Copper were not found in the bark of the *M. lucida*. Lead (Pb) was absent in crude leaf extracts of *M. lucida,* other minerals were present in appreciable quantity.

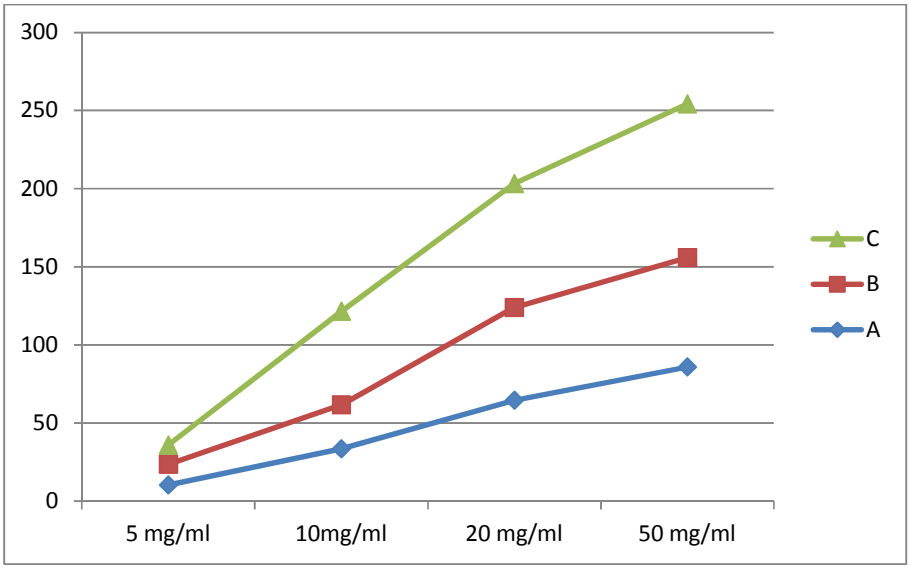

Fig. 1. Graphical representation of the standard used at various concentration for antioxidant determination (A, B, C.)

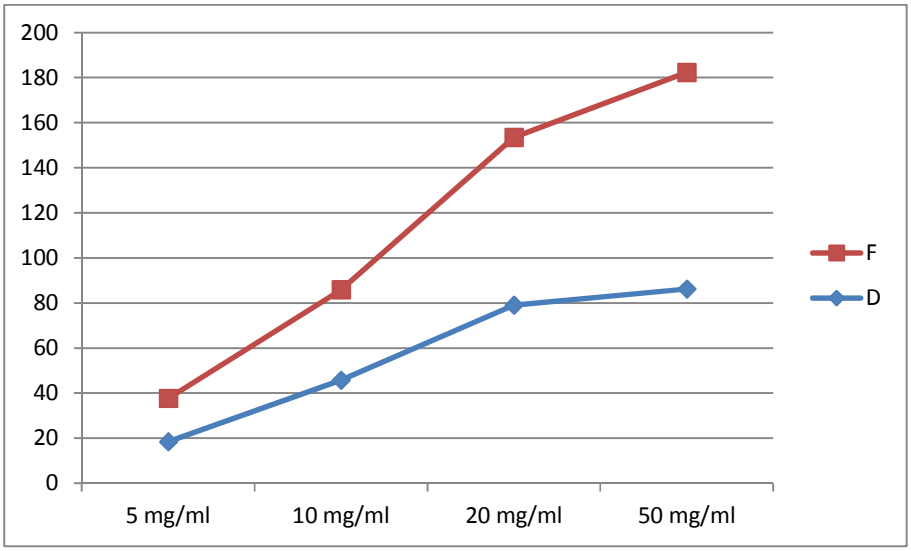

Fig. 2. Graphical representation of the standard used at various concentration for antioxidant determination (D&F)

Key; A-Phenol(Garlic acid); B-ABTS and DPPH(Ttolox Std); C-Flavonoids (Rutin std); D-Fe^{2+}(EDTA); E-FRAP, H_20_2, SO (VIT C)

Table 1. Quantitative chemical analysis of minerals present in *M. lucida* extracts (mg/100 g)

Sample	Na^+	K^+	Ca^{2+}	Mg^{2+}	Zn^+	Fe^{2+}	Pb^+	Cu^{2-}	Mn^{2-}	P^{2-}
M. lucida bark	20.33	41.24	15.33	25.38*	18.71	4.38	ND	ND	15.34	85.42
M. lucida leaf	20.01**	26.17	31.42	22.06	25.02	5.70	ND	0.01	6.20	25.12
M. lucida stem	19.82	24.77	29.49*	24.21	36.10	6.53	ND	0.02	5.45	35.78

*Difference between bark, leaf and stem were compared with one another. *P<0.05, **P<0.01, (Student's t-test)*
Key -ND =Not detected

Table 2. Composition of antinutrient present in *M. lucida* extracts in percentage (%)

Parameters	Leaf	Bark	Stem
Tannin	2.25**	3,45	2.45
Phenol	3.47	5.78	4.65
Phylate	12.42	1.25*	1.30
Oxalate	8.59	1.50	2.00
Saponin	7.60	9.34	8.23
Flavonoid	10.40	12.45**	7.72
Alkaloids	4.37	6.32	3.67

Difference between bark, leaf and stem were compared with one another. P<0.05, P<0.01, P<0.001 (Student's t-test). ND = Not detected

Table 2 shows the Quantity and composition of Anti- nutrient in *M. lucida* extracts in percentage. The Crude leaf extracts of *M. lucida* contains phytate and flavonoids, and it has the largest deposits of the anti-nutrient, which are 12,40% and 10,40% respectively. Flavonoids, alkaloids and saponin dominates the largest quantity composition found in the crude bark extracts of *M. lucida* which is 12,45%, 9.34%,and 5,78% respectively. The crude stem extracts of *M lucida* has the largest contents of saponin, flavonoids and phenol which are 8.23%, 7,72%, and 4.65% respectively.

Table 3 shows the proximate nutrient percentage composition of the crude extracts of *M. lucida* contain Carbohydrates, Moisture contents, Ash, fats, crude protein and fibre. It was observed that Carbohydrates, Moisture contents and Ash has the largest quantity the in the crude leaf and bark extracts of *M. lucida* with 53.74%, 16.19% and 8,75% respectively. The crude stem extract contain carbohydrate and moisture content.

Table 4 shows the In-vitro antioxidant of the ethyl acetate extracts of *M. lucida.* It was observed that the extract contain phenol, Abts (Azino-bis

(3-ethylbenthiazoline-6-sulphuric acid), Flavonoids, Fe2+, FRAP (Ferric Reducing Property), DPPH (Diphenyl -2-picryhydrazyl), H_20_2, and SO in 5, 10, 20 and 50 mg/ml concentration.

Table 5 shows the standard used for the antioxidant at various degree of concentration 5, 10, 20 and 50 mg/ml respectively.

4.1 Statistical Analysis

The contents were significantly higher in stem than the other parts (applicable to all tables with quantitative data). The value was considered significant at $P<0.05$.

5. DISCUSSION

Elemental composition, evaluation of anti-nutrients, and antioxidant potential of *M. lucida* extracts of (leaf, stem and bark) were investigated in the present study. It was observed that the plant contained many bioactive compounds. The bark and stem of the *M. lucida extracts* contains flavonoids and anthraquinones which are known antioxidants [20,21,40,41]. Flavonoids are known to be: anti-allergies, anti-inflammatory, free radical scavengers, hepatoprotector. Furthermore, flavonoids are known to be anti-microbial, anti-ulcer, anti-virus, tumors, and affecting platelet aggregation [20,21]. Flavonoids are known to inhibit specific enzymes as well. For example, flavonoids block the angiotensin-converting enzyme (ACE) that raises blood pressure. By blocking the "suicide" enzyme cyclooxygenase (that breaks down prostaglandins, they), flavonoids are reported to prevent platelet stickiness and platelet aggregation. Flavonoids are also known to protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells. *M. lucida* was also reported to contain alkaloids to be effective against pathogenic microorganism [40,37] It is worth mentioning that *M. lucida* extracts contained bioactive phenols [21,1,37]. Several phenols are known to have the ability to block specific enzymes that cause inflammation [42]. Phenol could modify the prostaglandin pathways and thereby protect platelets from clumping [43,42]. In earlier studies phenols demonstrated well the ability to block the uptake of cholesterol and facilitate its excretion from the body. Cholesterol has long been implicated as a significant risk factor in cardiovascular disease, thus justifying the traditional medicinal claims [43,39].

The activity due to different minerals identified in *M. lucida* also plays a major role in the prevention of infectious and communicable diseases. This is an important attribute of this medicinal plant. Minerals like Sodium (Na), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Lead (Pb), Copper(Cu), Manganese (Mn), Potasium (K) and Phosphorus (P) plays a major role in infectious diseases, the chemical balance of the human system and food preservation [12]. The toxic elements like Pb were absent in all parts of *M. lucida* under investigation.

The composition of anti-nutrient content (Tannin, Phenol, Phylate, Oxalate, Saponin, Flavonoid and Alkaloids) in leaf, bark and stem *M. lucida* extracts were also studied. Such compounds are known to have different biological activities including free radical scavenging and antioxidant potential which play a protecting role in the body [33,44,45,21,31]. There is increasing evidence that antioxidants derived from indigenous plant sources may be useful in preventing the deleterious consequences of oxidative stress [38,46]. Therefore, there is increasing interest in the protective biochemical functions with natural antioxidants present in spices, herbs and medicinal plants [10,47,40]. The proximate analysis of *M. lucida* were also taking into consideration during the course of this research work, it was observed that the plant contain Carbohydrates, Crude protein, Fats, Moisture content, Fibre, and ash in different composition justifying the plant to be edible and nutritious [33,45,48].

Table 3. Proximate nutrient composition of *M. lucida* extracts in percentage (%)

Samples	% Ash	% MC	% FAT	% CP	% Fibre	% CHO
M. lucida leaf	8.75	7.35	16.19	5.42	8.55	53.74
M. lucida bark	10.56	9.12	14.45	6.59	10.33	42.59
M. lucida stem	10.12	9.00	12.01	6.55	10.12	41.17

Key= MC = Moisture content; CHO = carbohydrate; CP = Crude protein

Table 4. *In-vitro* antioxidant assay of ethyl acetate extracts of *M. lucida* (mg/ml)

Concentration	Leaf				Stem				Bark			
	5 mg/Ml	**10 mg/ml**	**20 mg/ml**	**50 mg/ml**	**5 mg/ml**	**10 mg/ml**	**20 mg/ml**	**50 mg/ml**	**5 mg/ml**	**10 mg/ml**	**20 mg/ml**	**50 mg/ml**
Phenol	4.3563	8.74451*	13.1644	20.54r	5.64418	10.9385	16.56689	**20,763**	6.20065	12.4649	18.6542	**25.873**
Flavonoid	2.4270	5.4141	8.03525	9.454*	3.59874	7.42065	10.6846	15.456	4.1845	8.03439	12.0794	17.365
FRAP	10.7755	21.8691	31.3195	42.453	20.5439	41.6418	61.6319	70.652	27.7444	59.7980	85.0964	90.534
FE 2+	29.8850	41.5697	57.9501	62.432	37.5478	50.5747*	62.8352	72.873	35.4256	49.5210	64.0153	70.8762
H_2O_2%	12.6935	27.0897	46.4396	62.453	40.8668	57.2755	64.3962	70.352	31.8885	46.7492	59.9071	62.934
ABTS µ	13.0752	15.7548	18.3585	29.765	15.1277	17.8074	19.9929	30.978**	14.3675	16.8381	19.1947	31.923
DPPH%	11.5405	29.8648	40.2972	50.354	37.7027	40.7072	55.4594	60.672	38.5135	37.4054	48.9729	63.281
SO%	17.9775	33.5206	50.9363	69.863	38.2022	51.8726	55.678	61.463	30.3370	43.6329	57.8651	70,862*

Difference between bark, leaf and stem were compared with one another. P<0.05, P<0.01, P<0.001. (Student's t-test).
*Key: FRAP = Ferric reducing property; ABTS = Azino-bis (3-ethylbenthiazoline-6-sulphuric acid) (unit = **µ**); SO = Superoxide;*
DPPH = Diphenyl -2-picryhydrazyl (unit = %) and H_2O_2 = (unit = %)

Table 5. Concentration of sample standard used for antioxidant analysis

Conc. (sample STD)	A	B	C	D	F
5 mg/ml	10.35	13.38	12.26	18.45	19.23
10 mg/ml	33.5	28.2	59.87	45.78	40.12
20 mg/ml	64.6	59.34	79.35	79.06	74.43
50 mg/ml	85.9	70.02	98.31	86.23	96.09

6. CONCLUSION

This research work presented demonstrated well that different parts of *M. lucida* are biologically active and nutritious due to its chemical composition. The current findings are a step forward to increase knowledge and usefulness of *M. lucida* as a medicinal plant. The free radical scavenging potential and confirmation of the antioxidant activity and its nutritious value add to the importance of *M. lucida* and details studies are warranted.

7. RECOMMENDATION

It is thereby recommended that the medicinal plant such as *Morinda lucida* and other types of medicinal plants should be studied and exploited for future use.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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