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Phytochemical Screening, Nutrient Analysis and Antimicrobial Activity of the Leaves of *Lasianthera africana* and *Dennettia tripetala* on Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RUBE, NUA, CAE, UOE and CSO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors UOE and CSO managed the analyses of the study. Authors CSO and RUBE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The spate of resistance to conventional antibiotics is becoming worrisome. As alternatives, plants are investigated for bioactive components with antimicrobial activity. The aim of this study was therefore to investigate the nutrients and anti-nutrients composition, phytochemical components and antimicrobial activity of *Dennettia tripetala* and *Lasianthera africana* extracts on clinical isolates. The leaves were screened qualitatively and quantitatively for phytochemicals using aqueous and ethanolic extracts while the antimicrobial activity was done using standard microbiological techniques. The results of the phytochemical screening showed the presence of alkaloids, glycosides, reducing compounds and polyphenol in both plants. Quantification of the phytochemicals showed that polyphenol was the most abundant with values of 17.63% and 14.66% respectively, in *D. tripetala and L. africana.* Proximate composition in mg/100 g showed that protein was (19.86 - 5.70), fibre (10.80 -13.86), ash (3.16 - 9.28), carbohydrate (49.70 - 73.04)

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and fat (7.30 each) in both plants, respectively. The anti-nutrients composition in mg/100 g showed the presence of hydrocyanic acid (6.17 - 7.56), soluble oxalate (7.30 - 17.62), total oxalate (17.10-39.70) and phytate (10.19 - 13.20) in both plants. The antimicrobial activity showed that the aqueous and ethanolic extracts of both plants inhibited the growth of *Staphylococcus* sp, *Esherichia coli*, *Salmonella* sp, *Shigella* sp, *Bacillus* sp, and *Pseudomonas aeruginosa.* The highest inhibition (25 mm) was obtained with the ethanolic extract of *L. africana* on *P. aeruginosa*. The minimum inhibitory concentration (MIC) of the extracts ranged from 10 mg to 50 mg with *E. coli* and *Shigella* sp being the most inhibited. The findings of the study reveal that the plants are rich in nutrients, phytochemicals and have antimicrobial activity against isolates. Given the results in this study, there is a need to further exploit the antimicrobial potentials of these plants.

Keywords: Phytochemicals; medicinal plants; antimicrobial; anti-nutrients.

1. INTRODUCTION

Over the last few decades, the role of medicinal plants in the management of disease and health is increasing in demand for a number of reasons. Synthetic drugs of choice for treatment of infections are usually with side effects and there are increasing reports of the emergence of resistance which has prompted the increased search for novel and newer drugs [1]. A number of studies has shown that plants contains a plethora of phytochemicals in their organs which include alkaloids, tannins, saponins, cardiac glycosides, flavonoids, quninones, steroids, phytosteroids, terpenoids and polyphenols, and with excellent antimicrobial activity [2-3]. It is estimated that one fourth of the prescription dispensed from community pharmacies in the United State contains one or more plant derived ingredient (s) and their demand are already on the increase [4]. In India, the demand for medicinal plants and its products is estimated to be approximately one billion dollars per year [5]. It is estimated that 80% of the people living in developing world including Nigeria rely heavily on herbal medicine as an integral part of primary health care and traditional medicine [6].

Dennettia tripalata is a medicinal plant of the family annonaceae which bear edible fruits which are green when unripe and pink when ripe with a pungent spicy taste [7-8]. It is used in the treatment of cough, fever, diarrhea and rheumatism [9]. Older leaves of the plants are used as tea by locals. *L. africana* on the other hand, is a medicinal plant of the family icacinaceae used by the Ibibio and Anang tribes for the treatment of various ailments such as internal heat, urinary tract infection and enteric diseases [10].

The aim of this research is therefore to determine the phytochemical components, proximate composition, anti-nutrients and antimicrobial activity of the leaves extracts of *D. tripetala and L. africana.*

2. MATERIALS AND METHODS

2.1 Collection of Medicinal Plants

The leaves of *D. tripalata* and *L. africana* used in this study were collected in October 2015 from the farms of Indigenous farmers from Use-Offot Village with their approval. The leaves were identified by Mr. Frank Okpopoye of the University of Calabar Botanical Gardens, Cross Rivers State.

2.2 Collection and Identification of Clinical Isolates

The clinical isolates used in this study were obtained from stool, mid-stream urine, skin and wound swabs from patients attending St. Luke Hospital, Annua Offot, Akwa Ibom State, Nigeria. The numbers of stool, skin, urine and wound samples collected were six (6), four (4), eleven (11) and four (4), respectively. The bacterial isolates were characterized using morphological and biochemical tests as previously described [11-13]. They included gram staining, spore stain, motility test, and biochemical tests included catalase, citrate, coagulase, indole, oxidase and sugar fermentation, urease, methyl red, and voges proskauer. The probable isolates were *Esherichia coli, Pseudomonas aeruginosa, Salmonella sp, Shigella, Staphylococcus aureus, Staphylococcus sp*, and *Bacillus sp*.

2.3 Preparation of Plant Extracts

The leaves of *D. tripalata* and *L. africana* were oven dried for 1 hour at 100℃. After drying, the leaves were separately ground using a mortar and pestle, and stored in universal bottle for

extraction. The aqueous and ethanolic extracts were then prepared as previously described [2-3]. Briefly, 20 g of plant powder from each plant was extracted in 100 ml of distilled water and ethanol separately. These were then allowed to stand for 72 hours and then heated in a water bath at 70℃ to allow the solvents to evaporate resulting in the crude extracts. These were then stored at 4℃ until required for use.

2.4 Preparation of Steam Extract

Steam extract was prepared by the methods described by Pavia [14]. Briefly, approximately 3.5 g of the plant powder was weighed into a 100 ml round neck flask containing 40 ml of distilled water for distillation. The essential oil was extracted from the distillate (a mixture of water and oil) using diethyl ester with the aid of a separating funnel. The ethereal layer was dried with anhydrous magnesium sulphate and filtered while the ether removed using a rotating evaporator.

2.5 Phytochemical Screening of Plant Material

The plants were screened for phytochemicals such as tannins, saponins, polyphenols, anthraquinones, glycosides, flavonoids, alkaloids, reducing compounds, phlobatannins, and hydroxymethyl anthraquinones by methods previously described [2-3].

2.6 Proximate Composition of Plant Material

The plant materials were analyzed for proximate nutrient composition using the methods already described by the Association of Official Analytical Chemists [3-15]. These included moisture, ash, protein, fat and carbohydrate.

2.7 Moisture

About 5 g of freshly obtained leaves of both plants were dried in an oven to a constant temperature of 70℃. The moisture content was expressed as loss in weight obtained after cool weighing.

2.8 Ash Content

About 5 g of each of the dried plant material were placed in a crucible and heated to 550℃ in order to burn off the organic components. The crucible with its contents was cooled in a desiccator and weighed. The ash was then expressed as a percentage of the original dry weight of the sample.

2.9 Crude Protein

This was done using the micro-Kjedahl method. The protein nitrogen in 5 g of each of the plant sample was converted into ammonium sulphate by digestion with concentrated sulphuric acid in the presence of copper sulphate as a catalyst. The liberated ammonia was collected in boric acid double indicator solution. Nitrogen was the quantified via titration using standard HCl until end point was reached. The nitrogen was multiplied by a factor of 6.25 to derive the protein content.

2.10 Crude Fat

Crude fat was extracted from both plant samples using about 5 g of the plant samples, petroleum ether and Soxhlet extractor apparatus. The weight of the fat obtained after evaporating off the petroleum ether from the extract gave the crude fat in the samples and this was expressed as a percentage.

2.11 Crude Fibre

About 5 g of the defatted sample was used to determine the fibre contents in both plants using separate exhaustive extraction by acid digestion, filtration and base digestion. The acid digestion was done using 1.25% of H_2SO_4 while the base digestion was done using 1.25% of NaOH. Both steps were boiled for 30 minutes. Following the base digestion, the residues were washed off using 95% methanol. The residue was eventually ignited at 550℃. Fibre content was then expressed as a percentage using lost on ashing and initial weight.

2.12 Carbohydrate

The carbohydrate content was obtained as the difference from 100 of crude protein, fat, ash, and fibre.

2.13 Estimation of Anti-nutrients

The anti-nutrients examined were hydrocyanic acid, phytic acid and oxalate. These were done as previously described [16-17].

2.14 Hydrocyanic Acid (HCN)

Exactly 10 g of each of the plant sample was soaked in 300 ml in distilled water for about 4hours for the liberation of the cyanide. The liberated cyanide was steam distilled into 20 ml (2.5% w/v) NaOH. To the mixture, 8 ml of NH_4OH was added to the distillate before titrating with 0.02 M $AGNO_3$ to a faint and permanent turbidity. 1 ml of 0.02 $AgNO_3$ gave 1.08 mg of HCN.

2.15 Phytic Acid

Two grams of each of the samples was extracted with 0.5M HCl. Ferric chloride was used to precipitate the phytic acid to ferric phytate. NaOH solution was then used to convert the precipitate into sodium phytate and then digested with acid mixture containing equal portion of concentrated H_2SO_4 and $HClO_4$. The liberated phosphorus was then quantified colorimetrically at 620 nm after colour development with molybdate solution.

2.16 Oxalate

About 2.5 g of the sample was extracted with dilute HCl. The oxalic acid in the extract was precipitated with calcium chloride as calcium salts. The precipitated oxalate was washed with 25% H_2SO_4 and dissolved in hot water before titrating with $KMnO_4$.

2.17 Antimicrobial Sensitivity Test

The antimicrobial sensitivity test was carried out using agar disk diffusion methods previously described [18-19]. Briefly, a manual borer was used to prepare 5mm of sensitivity disk from Whatman filter paper No1; the discs were heated in an oven for 30 minutes at 100℃ to sterilize them. After sterilization, these discs were soaked in each of the extracts (aqueous and ethanolic) for 15 minutes and were gently placed on Muller Hinton Agar plates inoculated with the test organisms. The plates were then incubated at 37℃ overnight. Following incubation, the diameters of the zones of inhibitions were measured in millimeter.

2.18 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plants extracts were determined using pour plate technique. Different concentration of 10 mg, 20 mg, 50 mg and 100 mg were prepared by weighing out 1.0 g, 2.0 g, 5.0 g and 10.0 g of each of the extracts and dissolved in 1ml of water separately. Sterilized 5 mm discs were soaked in the various concentrations for 10minutes and allowed to air dry. These were then incubated with the pour plates of the test organisms at 37℃ for 24 hours. The plates were then examined for inhibition and the lowest inhibitory concentration taken as the MIC for the extract and test organism.

2.19 Statistical Analysis

Replicate readings obtained were analyzed for significance using Analysis of Variance (ANOVA) at 95% significance level. All the analyses were done using Microsoft Excel 2007 Version.

3. RESULTS

The results of the study are presented in the figure and tables below. Locally, the plants are known by different names by some tribes in Southern Nigeria as presented in Table 1. The isolates gotten from the samples were *Esherichia coli, Pseudomonas aeruginosa, Salmonella species, Shigella species, Staphylococcus aureus, Staphylococcus species* and *Bacillus species*. The frequencies of occurrence of these isolates in the different samples are as shown in Fig. 1. The results of the qualitative and qualitative phytochemical screenings are presented in Tables 2 and 3. Reducing compounds and polyphenols were present in excess or much excess in the extracts. Polyphenol was the most abundant on quantification. Table 4 and 5 show the results of the proximate composition and anti-nutrients. After moisture, the next most abundant nutrient was carbohydrate. The results of the susceptibility of the isolates to the ethanolic and aqueous extracts and minimum inhibitory concentrations are presented in Tables 6 and 7. The highest zone of inhibition of 25.00 mm was observed against *E. coli* using ethanolic extracts of *L. africana*. At 50 mg and 100 mg, there were no growths.

4. DISCUSSION

L. africana locally called “editan” is a delicious vegetable whose green soft leaves are widely consumed as vegetable in soups majorly by the Ibibio speaking tribes in the Niger Delta region of Nigeria. In addition, it is used as an antianalgesic, laxative, antidiabetic, antipyretic,

and antimalarial therapy [20]. Proximate composition analysis carried out in our study showed that *L. africana* is a rich source of nutrients. In another study [21] the proximate composition of *L. africana* was found to be 77.67% moisture, 63.92% carbohydrate, 7.06% lipid, 15.04% protein, 5.12% fibre, and 8.50% ash. Compared to our study, moisture, lipid and ash were almost similar, protein and fibre was higher while carbohydrate was lower.

Table 1. Plant used in the study

S/N	Scientific name	Family name	Local name	Part used
1	*Dennettia* tripetala (P. Beauv).	Annonaceae	Nakrika/Mmimi/Igberi (by Ibibio/Anang, Igbo and Yoruba tribes, respectively).	Leaves
2	*Lasianthera africana* (G. Baker)	Icacinaceae	Editan (by the Ibibio and Efik tribes).	Leaves

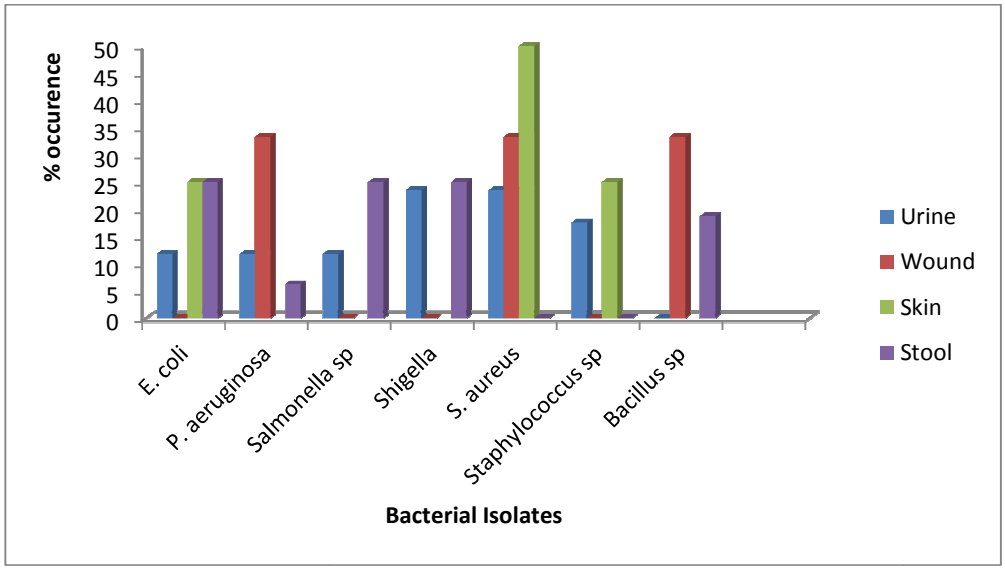

Fig. 1. Frequency of occurrence of bacterial isolates from the various samples

Table 2. Qualitative assay of phytochemicals and essential oil

Phytochemicals	*D. tripetala* Eth extract	Aq. extract	Steam extract	*L. africana* Eth extract	Aq. extract	Steam extract
Alkaloids	+	+		++	+	
Glycosides	+	+		++	++	
Saponins	-	-		++	+	
Tannins	-	-		++	+	
Flavonoids	-	+		+	++	
Reducing Compounds	++	+++		++	++	
Polyphenols	+++	+++		+++	++	
Phlobatannins	-	-		-	-	
Anthraquinones	-	-		-	-	
Hydroxymethyl anthraquinones	-	-		-	-	
Essential oil			+			+

Key: + = present, ++= present in excess, +++ = present in much excess, - = absent, eth= ethanol and aq= aqueous

Table 3. Quantitative estimation of phytochemicals (Mean±SD)

Phytochemical	*D. tripetala*	*L. africana*
Alkaloids	1.40±0.06[a]	2.70±0.06
Glycosides	1.31±0.01	1.40±0.01
Saponins	-	3.32±0.12
Tannins	-	0.46±0.01
Flavonoids	3.10±0.06	8.10±0.06
Reducing compounds	4.59±0.01	7.19±0.01
Polyphenols	17.63±0.01	14.66±0.01

[a]*Replicate readings show significance (p< 0.01)*

Table 4. Proximate composition of plants under study (g/100 dry matter)

Proximate composition	*D. tripetala*	*L. africana*
Moisture	78.75±0.01[b]	76.62±0.01
Ash	3.16±0.01	9.28±0.01
Protein	5.70±0.06	19.86±0.06
Fat	7.30±0.06	7.30±0.06
Fibres	10.80±0.06	13.83±0.01
Carbohydrates	73.04±0.01	49.70±0.01

[b]*Replicate readings show significance at (p< 0.01)*

Table 5. Anti-nutrient (toxicants) Analysis of *D. tripetala* and *L. africana* (mg/100 g)

Proximate composition	*D. tripetala*	*L. africana*
Hydrocyanic acid	7.56±0.01[c]	6.17±0.01
Soluble oxalate	17.62±0.01	7.30±0.06
Total oxalate	39.70±0.06	17.10±0.06
Phytate	10.19±0.01	13.20±0.06

[c]*Replicate readings show significance at (p< 0.01)*

Table 6. Antibacterial susceptibility of the ethanolic, and aqueous of *D. tripetala* and *L. africana* (mm) (Mean±SD)

Bacterial Isolates	*D.*	*Tripetala*	*L.*	*Africana*
	Eth	Aqu	Eth	Aqu
Bacillus sp	13.00±1.40	9.00±0.00	23.70±0.40	20.30±1.90
E. coli	10.70±0.80	15.30±1.20	25.00±3.10	20.00±1.40
P. aeruginosa	10.30±1.50	8.30±0.80	14.00±2.60	13.30±1.50
Salmonella sp	12.30±0.80	9.70±0.80	20.70±0.80	18.00±1.40
Shigella sp	13.00±2.10	14.70±0.80	18.70±0.80	23.00±1.20
S. aureus	-	-	8.70±0.80	14.30±0.80
Staphylococcus sp	15.70±0.70	11.30±0.8	10.60±0.40	18.30±0.80

Key: Eth = Ethanol and Aqu = Aqueous

On the other hand, *D. tripetala* popularly called pepper fruit is a fruit tree that is widely distributed in Southern Nigeria. Like *L. africana*, the leaves as well as the fruits are used although not as vegetable but as spices [22]. The proximate composition of the leaves of *D. tripetala* in our study showed that it contains 78.85% moisture, 3.16% ash, 5.70% protein, 7.30% fat, fibre 10.80% and carbohydrate 73.04%. Carbohydrate was higher than that in *L. africana*, fat and moisture content were similar while the rest were lower. In an earlier study by Ihemeje et al. [23] on the unripe and ripe fruits of *D. tripetala*, as expected the moisture content was far lower in the fruits than the leaves. The protein content of the leaves was in between the unripe and ripe fruits while carbohydrate was lower than in both fruits. The fibre and ash in our study was however lower.

Further analysis revealed the presence of anti-nutrients such as hydrocyanic acid, total oxalate, soluble oxalate and phytate. In both plants total oxalate was the highest in concentration with mean values of 39.70 and 17.10 mg/100 g for *D. tripetala* and *L. africana,* respectively.

Table 7. Minimum inhibitory concentration (MIC) of ethanolic and aqueous extracts of the study plants

Bacterial isolates	Extracts	10 mg	20 mg	50 mg	100 mg
D. tripetala					
Bacillus sp	Ethanolic	-	-	+	++
E. coli	Aqueous	-	-	+	++
Salmonella sp	Ethanolic	-	-	+	++
Shigella sp	Ethanolic	-	-	+	++
Shigella sp	Aqueous	-	-	+	++
Staphylococcus sp	Ethanolic	-	-	+	++
L. africana					
Bacillus sp	Ethanolic	-	+	+	+++
Bacillus sp	Aqueous	-	+	++	+++
E. coli	Ethanolic	+	++	+++	+++
E. coli	Aqueous	-	+	++	++
P. aeruginosa	Aqueous	-	-	+	++
Salmonella sp	Ethanolic	-	+	++	++
Salmonella sp	Aqueous	-	+	++	++
Shigella sp	Ethanolic	-	+	++	+++
Shigella sp	Aqueous	+	++	+++	+++
S. aureus	Aqueous	-	-	+	++
Staphylococcus sp	Ethanolic	-	+	++	++

Key: = - growth, + = minimum inhibitory concentration, ++ = high zones of inhibition and = +++ very high zones of inhibition.

Hydrocyanic acid was the least anti-nutrient in both plants. The total oxalate reported in our study was far less than that reported in the seeds and skins of ready to eat green and golden Kiwifruit in New Zealand [24]. However, soluble oxalate for *D. tripetala* was slightly higher while that of L*. africana* was lower. In another study by Alexander et al. [25] they found the concentration of HCN in cassava to be in the range of 300 to 2,360 (mg/kg) which is far less than what we found in our study plants.

The amounts of all the anti-nutrients examined in our study were equally less than that reported for commonly eaten vegetables such as *Vernonia amygdalina* (Bitter leaf), *Cochorous olitorius* (Ewedu), *Talinum triangulare* (Water leaf), *Moringa oleifera* (Drum stick) and *Telfariria occidentalis* (Pumpkin leaf) by Agbaire [26] and Agbaire and Emoyan [27].

Phytochemical screening of both plants revealed the presence of the following phytochemicals: alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds and polyphenol but not phlobatannins, anthraquinones and hydroxymethyl anthraquinones in the ethanol and aqueous extracts of *L. africana.* Saponins, tannins and flavonoids were not found in the ethanolic extract of *D. tripetala* however, it had flavonoid in the aqueous extract. The most abundant phytochemical in both plants qualitatively and quantitatively was polyphenol.

Okoronkwo et al. [28] and Osuagwu and Eme [29] confirmed the presence of alkaloid, phenol, saponin, tannin and flavonoid in *D. tripetala*. *However,* we did not detect saponin and tannin. Furthermore, on quantification, the most abundant was alkaloids while in our study the most abundant was polyphenol. The next abundant phytochemical was flavonoids and reducing compounds which were more in *L. africana* and *D. tripetala*.

The isolates used in this study were gotten from clinical samples such as skin, stool, urine and wound. When the isolates were subjected to sensitivity testing using the extracts, the test isolates were inhibited differently by the various extracts. The lowest inhibition (8.30 mm) was seen with the aqueous extract of *D. tripetala* on *P. aeruginosa* and the highest inhibition (25.00 mm) was observed with *E. coli* with *L. africana*. The *L. africana* extracts were far better than the *D. tripetala* on the test isolates. The zones of inhibition recorded for *D. tripetala* in our study were almost similar to that recorded by Osuagwu and Eme [29]. Okoronkwo et al. [28] also reported zones of inhibition and were slightly lower than that our findings. The observed inhibitory activity of these plants leaves have

been used to explain why they are used in traditional practice and it is on the increase [30]. Okoronkwo et al. [28] using gas chromatography coupled with mass spectrometry showed that chloroform extracts contains five alcoholic compounds out of the seventeen compounds it contained. The high prevalence of alcoholic compound explains the high percentage of polyphenols that was found in *D. tripetala* in our study and could also explain the zones of inhibitions observed in our study.

Studies have shown that all four varieties of *L. africana* are rich in phytochemical and have been used as a medicinal plant [2,20]. Andy et al. [10] and Okokon et al. [20] reported the use of this plant for the treatment of ailments such as typhoid fever, diarrhea, candidiasis, constipation and stomach ache, and they also have antimicrobial and antidiabetic properties. Our study confirmed that it is rich in a plethora of phytochemicals as seen in Tables 2 and 3. Our zones of inhibition on the test isolates are comparable to those of Andy et al. [9]. The MIC for *D. tripetala* was 50 mg/ml for all the test isolates while *L. africana* was 10 mg/ml for *E. coli* and *Shigella* sp using ethanolic and aqueous extracts, respectively. These were much lower than those earlier reported.

5. CONCLUSION

The present study reveals the presence of nutrients, phytochemicals, allowable limits of anti-nutrients and antimicrobial activity of the leaves of *D. tripetala* and *L. africana*. However, more research is needed to establish the actual bioactive component or components responsible for these activities if these extracts are to be formulated into conventional medicines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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