Phytoconstituents profiling of Selaginella willdenowii (Desv.) Baker and Pharmacological Potential

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RESEARCH ARTICLE

Hippobroma longiflora (L.) G. Don: Comparative Phytochemical Screening and Potential Activities of Flower and Leaf by GC-MS

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ABSTRACT:
Hippobroma longiflora (L.) G. Don or "*kitolod*" (Indonesia) is widely known as a traditional food medicine. However, the bioactive constituents of this plant are still unclear. Therefore, we investigated the bioactive compounds of *H. longiflora* (leaves and flowers) with Gas Chromatography-Mass Spectrometry (GC-MS) and further evaluated their potential biological activity. Ethanol extracts from the flowers and leaves of *H. longiflora* are targeted for phytochemical profiling of this plant. As a result, 16 metabolite compounds on leaves and 21 on flowers were successfully detected. A total of seven compounds, namely 2, 6, 10, 14, 18, 22-. Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (all--E)-, 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne, Stigmast-5-EN-3-OL, Stigmasterol, and Vitamin E dominate in both parts of this plant. The results of phytochemical analysis at the National Institute of Standard and Technique (NIST), *H. longiflora* can be an essential source of anti-tumor, anti-cancer, antibacterial, and antioxidant drugs.

KEYWORDS: Bioactive constituents, *Hippobroma longiflora* (L.) G. Don, GC-MS, kitolod, phytochemicals.

INTRODUCTION:

Medicinal plants contain traditional bioactive compounds beneficial to humans in curing various diseases[1]. Medicinal plants have been used since ancient times[2] as a tonic, food, or to increase immunity and body strength[2–5]. It has the characteristic of a thin and low rosette[6,7]. Green leaves with toothed linear and curved growth and white flowers with elongated tubular [8]. The plant belongs to Campanulaceae and spreads in tropical, temperate, and cold regions[9,10].

The flower and leaf parts of *H. longiflora* (L.) G. Don is commonly used in medicine and food. The ethanol extract of *H. longiflora* (L.) G. Don leaves alkaloids, flavonoids, polyphenols, monoterpenoids, sesquiterpenoids, quinine, steroids, coumarins, tannins, and saponins[11–13]. The compound (−)-Lobeline is known that have been isolated from *Lobelia inflata*, extensively studied its structure and biological activity[14], and is thought to have many functional similarities with the genus Hippobroma[15].

Bioactive compounds in *H. longiflora* (L.) G. Don flower extract is a group of fatty acids and their derivatives[16].

Pharmacologically, the flavonoid content of *H longiflora* (L.) G. Don can be used as a natural sunscreen and inhibit the development of cataracts[17–19]. Other uses of *H. longiflora* (L.) G. Don is also an antioxidant, anti-cancer, anti-inflammatory, antibacterial, anti-diabetic, antimalarial, anti-tumor, antimicrobial, antifungal, anti-insecticide, and antiseptic[20–22]. Other studies prove that the bioactive constituents of *H. longiflora* (L.) G. Don can be used as a folk remedy for bronchitis, laryngitis, asthma, wounds, eye medication, anti-neoplastic, hemostasis, and analgesic[23,24].

Recent reports state that a 75% concentration of ethanol extract from *H. longiflora* (L.) G. Don leaves effectively inhibit the growth of *Staphylococcus aureus* and *Salmonella typhi* with robust criteria[25,26]. Other studies also mentioned that ethanol extract from *H. longiflora* (L.) G. Don leaves inhibits corneal neovascularization after seven-day administration to the eyes that have experienced chemical trauma due to the pesticide Rotraz© 200EC[27]. *H. longiflora* (L.) G. Don is documented in Indonesian pharmacology as one of the

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traditional herbs (JAMU) by the Indonesian population. They are traditionally extracted by the natives to treat human diseases. However, the complete constituents of this plant are still unclear. Hence, this study aims to compare the phytochemical constituents of *H. longiflora* (L.) G. Don (leaves and flowers) with GC-MS analysis and further evaluate the potential for biological activity.

MATERIALS AND METHODS:

Plant Materials:

Fresh plants of *H. longiflora* (L.) G. Don a were collected from farmers' gardens in Mustika Jaya District, Bekasi, West Java, Indonesia (6°17'31"S, 107°1'46"E) in August 2022. This plant is authenticated at ELSA, Indonesia Botani Identification Services, and specimens are deposited at the university with voucher number 1961537. The sample was washed and stored at 20°C until subsequent analysis.

Sample Extraction Preparation:

Each part was weighed by 100g and oven dried at 33.5°C for 45 minutes following procedure [28]. Dried samples were mashed with a grinder and stored at -18°C in low-density polyethylene bags[29]. For three days, the stock concentration of 20mg/mL extract was prepared by soaking a powder sample of 20g of powder (40 mesh) in Pro Analyst Ethanol (99.8%). The solution mixture is stirred and placed in a 40°C waterbath and shaken at 250rpm for 1hour[30]. The mixture was centrifuged (2000rpm) for 10min, followed by filtration. The residue was immersed in a solvent and evaporated at 65°C using Rotary Evaporator. The extraction results are stored inside the fume hood to evaporate the excess solvent further until it produces a stickier and more concentrated extract. The extract was held at 4°C until further analysis[31].

GC-MS Condition:

Gas Chromatography-Mass Spectrometry (GC-MS) was used to decompose the phytoconstituents of target plants [32]. A combination of a gas chromatograph 7890 A (Agilent 19091-433HP, USA) and a mass spectrophotometer equipped with an HP-5 MS molten silica column connected to a 5675C-MS-D and a Three Axis detector was implemented[33]. The capillary column of GC-MS HP-5MS (30m 0.25mm ID 0.25m) consists of 5% diphenyl and 95% Dimethylpolysiloxane[28]. The ethanol extract was filtered using a 5µL filter in split mode (8:1). Helium gas was used as the carrier at a rate of 1.2mL/min at an injector temperature of 250°C. Then the analyte is separated into a silica capillary column. The oven program is set up as follows: an initial temperature of 80°C which is directly raised by 3°C/min to 150°C and held for 1 minute, then increased by 20°C/min to 280°C and held for 26minutes[28]. Ionization energy (70eV) was used to determine the mass spectrum.

Data Analysis:

The Agilent Mass Hunter software analyzes bioactive compounds by comparing mass fragments and the standard mass spectrum. Biological activity data were analyzed using the PubChem library database, Chemistry WebBook, and FOODB from the Metabolomics Innovation Center. This library was also used in Guang-Mei Tang's research in 2022[34].

RESULTS AND DISCUSSION:

This study shows that the GC-MS method is a powerful combination of phytoconstituents composition analysis [5,35–37]. The components can be separated effectively by GC and MS completing thorough component identification[12,38–41]. We agree that the GC-MS method provides an excellent opportunity to explore phytocomponents as has been done by many previous researchers[26,28,50,42–49]. This study used the GC-MS to determine the phytoconstituents of *H. longiflora* (L.) G. Don leaf and flower extracts. The GC-MS chromatogram of *H. longiflora* (L.) G. Don recorded a total of 37 peaks corresponding to phytochemical compounds recognized by attributing Retention Time (RT) and Concentration (%). As a result, as many as 16 unique metabolite compounds were present in the leaf and 21 in the flower of *H. longiflora*. The results of the leaf chromatogram of *H. longiflora* (L.) G. Don showed the highest RT value in Squalen from the Prenol lipids group (Figure 1).

-3,7,11,Trimethyl-dodeca-2,4,6,10-Tetraenal- is a unique compound that is only present on leaves with two different time retention. This lipid prenol derivative is a product of lycopene oxidation [51] in biological tissues and has been studied to have a role as an anti-depressant. Another unique compound detected that also has two retention times is 12-Methoxy-18-Nor-5.Beta.-Podocarpa-8,11,13-Trien-3.Alpha. O.L., a derivative of the Hydrophenanthrenes group, has yet to be discovered in terms of its benefits. Interpretation of the mass spectral chromatogram of *H. longiflora* (L.) G. Don compounds is presented in **Table 1.**

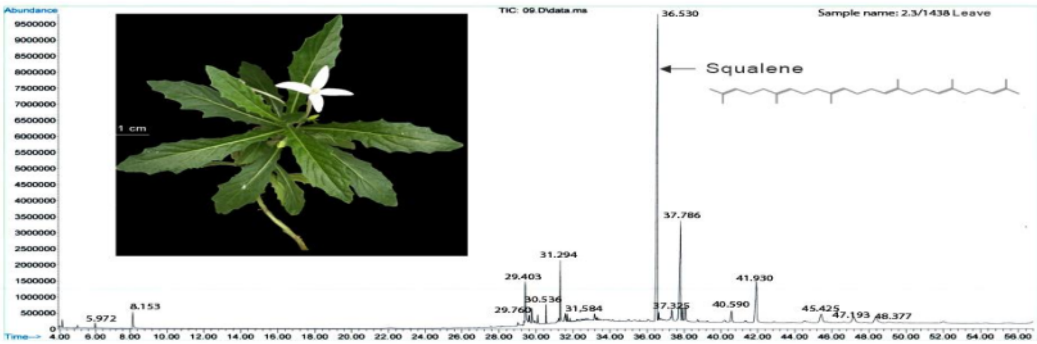

Figure 1: Chromatogram of *H. longiflora* (L.) G. Don leaf

Table 1: List of compounds leaf and flower of *H. longiflora* (L.) G. Don

M.F.	Compound	M.W.	Classification	Leaf		Flower	
				R.T.	% of Area	R.T.	% of Area
C_7H_6O	Benzaldehyde	106.12	Benzene and substituted derivatives	-	-	5.866	1,2
$C_{20}H_{40}O$	(2E)-3,7,11,15--Tetramethyl-2-Hexadecene--1-OL	296.5	Prenol lipids	31.296	4,17	-	-
$C_{26}H_{43}N_5O_4$	(E)-9-Octadecenoic Acid	489.7	Fatty acyls	31.585	1,17	-	-
$C_{15}H_{16}O_2S$	12-Methoxy--18-Nor-5.Beta. --Podocarpa-8,11,13-Trien-3.Alpha.-OL	260.4	Hydrophenanthrenes	- -	- -	37.370 38.260	1,89 2,62
$C_6H_5COCH_3$	1-Phenylethanone	120.15	Organooxygen compounds	8.155	1,65	-	-
$C_{18}H_{38}$	2,6,10-Trimethyl, - 14-Ethylene-14-Pentadecne	278.5	terpenoid	29.399	5,64	29.399	1,12
$C_{15}H_{16}O_2S$	2,4',6-Trimethyldiphenylsulfone	260.4	Not Found	37.784	14,81	-	-
$C_{30}H_{50}$	2,6,10,14,18,22--Tetracosahexaen, 2,6,10,15,19,23--hexamethyl-,(all-E)-	410.7	Prenol lipids	36.529	39,01	36.515	7,54
$C_9H_{10}N_2O_4$	2-[4-(-1 Methylethoxy) bnztl]-4,6-pyrimidinediol	210.19	Benzene and substituted derivatives	37.895	1,5	-	-
$C_{18}H_{12}O_2$	2-Hydroxy-2-Phenyl-1-Acenapthenon	260.29	Naphthalenes	37.322	1,85	-	-
$C_{15}H_{22}O$	3,7,11, -Trimethyl-dodeca-2,4,6,10-Tetraenal	218.33	Prenol lipids	29.613 29.758	1,24 1,82	- -	- -
$C_{30}H_{48}O$	4,4,6a,6b,8a,11,12,14b,Octamethy-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14,14b-Octadehydro-2H-Picen-3-one-	424.7	Prenol lipids	-	-	49.272	2,27
$C_{19}H_{22}O_6$	6,7,8,9-Tetrahydro--3-Methoxymethyl-5,9-Dimethyl-8-Hydroxy-Naptho [1,2-B] Furan	346.4	Naphthofurans	38.067	2,34	-	-
$C_{17}H_{12}N_2O$	6H-Pyrido- [4,3-B] Carbazole-5-Carboxaldehyde, 11-Methyl-	260.29	Carbazoles	-	-	38.032	41,3
$C_{10}H_{18}$	8-Methyl--7-Thiabicyclo [4.2.1] nonan	138.25	Not Found	-	-	43.018	1,33
$C_{39}H_{70}O_6$	9,12-Octadecadienoic Acid	635.0	Fatty Acyls	-	-	36.143	1,08
$C_{10}H_{21}NO$	Acteamine, N-(1,1,3,3-tetramethyl butyl)-	171.28	Indoles and derivatives	-	-	38.825	38.825
C_8H_8O	Acetophenone	120.15	Organooxygen compounds	-	-	8.127	5,04
$C_{30}H_{50}O$	Alpha Amyrin	426.7	Prenol lipids	48.375	2,23	-	-
$C_{31}H_{64}$	Hentriacontane	436.8	Saturated hydrocarbons	-	-	40.701	3,87
$C_{20}H_{42}$	Hexadecane, 2,6,10,14-Tetramethyl-	282.5	Diterpenes	-	-	42.873	2,45
$C_{20}H_{42}$	Icosane	282.5	Saturated hydrocarbons	-	-	35.040	1,85
$C_{29}H_{60}$	Nonacosane	408.8	Saturated hydrocarbons	-	-	37.288	1,89
$C_{19}H_{40}$	Nonadecane	268.5	Saturated hydrocarbons	-	-	32.261	1,76
$C_{28}H_{58}$	Octacosane	394.8	Saturated hydrocarbons	40.591	1,93	-	-
$C_{29}H_{50}O$	Stigmast-5-EN-3-OL-	414.7	Steroids and steroid derivatives	47.196 -	2,23 -	47.548 51.078	3,54 1,44
$C_{29}H_{50}O_2$	Vitamin E	430.7	Prenol lipids	41.928	8,09	42.038	4,77
$C_{29}H_{48}O$	Stigmasterol	412.7	Steroids and steroid derivatives	45.424	2,55	45.672	2,64
$C_{16}H_{30}O$	Z,Z-11,13-Hexadecadien-1-ol	238.41	Fatty acid derivative	-	-	32.516	1,57

Table 2: Equation of compounds in extracts of leaves and flowers of *H. longiflora* (L.) G. Don

Compounds	Leaf		Flower	
	R.T.	% of Area	R.T.	% of Area
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-	36.529	39,01	36.515	7,54
2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne	29.399	5,64	29.399	1,12
3,7,11,Trimethyl-dodeca-2,4,6,10-Tetraenal	29.613	1,24	-	-
	29.758	1,82	-	-
12-Methoxy-18--Nor-5.Beta.-Podocarpa-8,11, -13-Trien-3.Alpha.OL	-	-	37.370	1,89
	-	-	38.260	2,62
Stigmast-5-EN-3-OL	47.196	2,23	47.548	3,54
	-	-	51.078	1,44
Stigmasterol	45.424	2,55	45.672	2,64
Vitamin E	41.928	8,09	42.038	4,77

The main compounds in the leaf extract found with a high percent peak area are 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- or Squalene compounds (39.01%), 2,4',6-Trimethyldiphenylsulfone (14.81%), Vitamin E (8.09%), and 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne (5.64%), (2E)-3,7,11,15-Tetramethyl-2-Hexadecene-1-OL- (4.17%). There are several compounds with almost the same peak area, namely Stigmasterol (peak area: 2.55%), -6,7,8,9-Tetrahydro-3-Methoxymethyl-5,9--Dimethyl-8-Hydroxy-Naptho-[1,2-B] Furan (2.34%) and two compounds with the same peak Stigmast-5-EN-3-OL and Alpha Amyrin (2.23%).

The compound -6H-Pyrido [4,3-B] -Carbazole-5-Carboxaldehyde, 11-Methyl- belongs to the class Carbazoles and is the main phytoconstituent in the flower *H. longiflora* (L.) G. Don, indicating the presence of a dominant influence of function on the flower. Other compounds with a reasonably high peak are Vitamin E (4.77%) and hentriacontane (3.87%). The rest of the compound was found with almost the same peak area. 9,12-Octadecadienoic acid (1.08%) was found to be the compound with the lowest biological activity in the extracted compound in *H. longiflora* (L.) G. Don flowers. The nonane 8-Methyl-7-Thiabicyclo [4.2.1] compound is a new compound that cannot be detected in the database.

From a total of 37 compounds isolated from *H. longiflora* (L.) G. Don leaf and flower extracts, there are 7 compound variant equations with different percentages and retention times (**Table 2**). Compounds -2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)-, 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne, Stigmast-5-EN-3-OL, Stigmasterol, and Vitamin E are bioactive compounds found in flowers as well as leaves. The retention time of compounds -2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-,(all-E)- on the leaves are not much more significant when compared to the same compound in the flower part, and this suggests that the leaves take longer to come out. The compound also has a high percentage of areas in the leaf part. As for the compound 2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne has the same retention time on the leaf part of the flower. The repeated retention time belongs to the compound Stigmast-5-EN-3-OL; in the leaves, this compound has only one-time retention, but in flowers, it has two-time retention. The bioactive constituents of stigmasterol and Vitamin E indicate a stable retention time in the leaves and flowers of *H. longiflora* (L.) G. Don. The compound -3,7,11,Trimethyl-dodeca-2,4,6,10-Tetraenal was found only on the leaves alone but had a different time interval to exit the column and reach the detector, while 12-Methoxy-18-Nor-5.Beta.-Podocarpa-8,11,13-Trien-3.Alpha. O.L. is only found in the flower section and has a different retention time.

Various potentials are found in the content of leaf extracts and flowers of *H. longiflora* (L.) G. Don (Table 3). Squalene or by other names 2,6,10,14,18,22-Tetracosahexaen, -2,6,10,15,19,23-hexamethyl-,(all-E)-(peak area: 39,01%) is a natural triterpene hydrocarbon. It is an essential intermediary in intensifying many bioactive secondary metabolites[52]. This compound has various benefits for -human- health, such as anti-cancer effects, cardiovascular disease, and boosting the immune system[53]. In addition, it also has antibacterial properties [54].

In *H.longiflora* (L.) G Don flowers, there are bioactive compounds, namely -6H-Pyrido [4,3-B] Carbazole-5-Carboxaldehyde, 11-Methyl- (41.3%) or with the compound name ellipticine. This bioactive constituent is a pyridocarbazole type plant alkaloid that exhibits cytotoxic activity against tumor cells. It is known that ellipticine has exciting photophysical properties in different solvents[55]. It has pharmacological potential as an anti-tumor and anti-neoplastic[56]. Anti-neoplastic agents are drugs that prevent, kill, or inhibit the growth and spread of cancer cells. Most anti-neoplastic agents act on molecular targets important for cell division[57].

Then it was found that there were eight compounds in flowers and leaves whose biological activity was unknown. Compound -12-Methoxy-18-Nor-5.Beta.-Podocarpa-8,11,13-Trien-3.Alpha.-OL, 8-Methyl-7-Thiabicyclo- [4.2.1] nonan, 9,12-Octadecadienoic Acid, Hexadecane, 2,6,10,14-Tetramethyl-, Nonadecane on flowers has not found its biological activity. Other compounds in the leaves that until now are still unknown in biological activity are 2-[4-(-1 Methylethoxy) bnztl]-4,6-pyrimidinediol, 2-Hydroxy-2-Phenyl-1-Acenapthenon, -6,7,8,9--Tetrahydro-3--Methoxymethyl-5, -9-Dimethyl-8-Hydroxy-Naptho [1,2-B] Furan.

Table 3: Biological activities of *H. longiflora* (L.) G. Don

Compounds	Part	Biological Activity
Benzaldehyde	Flower	Flavoring agents [58] and irritants [59]
(2E)-3,7,11,15-Tetramethyl-2-Hexadecene-1-OL	Leaf	Antioxidant [60], anti-infective and antiparasitic [61]
(E)-9-Octadecenoic Acid	Leaf	Antifungal [62]
12-Methoxy-18-Nor-5.Beta.-Podocarpa-8,11,13-Trien-3.Alpha.-OL	Flower	Not Found
1-Phenylethanone	Leaf	Anti-diabetic and anti-adipogenic [63]
2,6,10-Trimethyl, 14-Ethylene-14-Pentadecne	Leaf and Flower	Antioxidant, Antiproliferative, and Anti-cancer [64]
2,4',6-Trimethyldiphenylsulfone	Leaf	Antibacterial and anti-cancer [65]
2,6,10,14,18,22-Tetracosahexaen, 2,6,10,15,19,23-hexamethyl-,(all-E)-	Leaf and Flower	Antibacterial [54] and insecticide [66]
2-[4-(-1 Methylethoxy) bnztl]-4,6-pyrimidinediol	Leaf	Not Found
2-Hydroxy-2-Phenyl-1-Acenapthenon	Leaf	Not Found
3,7,11, Trimethyl-dodeca-2,4,6,10-tetraenal	Leaf	Anti-depressants [67]
4,4,6a,6b,8a,11,12,14b,Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-Octadehydro-2H-Picen-3-one-	Flower	Anti-cancer [68]
6,7,8,9-Tetrahydro-3--Methoxymethyl-5,9-Dimethyl-8-Hydroxy-Naptho [1,2-B] Furan	Leaf	Not Found
6H-Pyrido [4,3-B] - Carbazole-5-Carboxaldehyde, 11-Methyl-	Flower	Antitumor dan antineoplastic [56]
8-Methyl-7-Thiabicyclo [4.2.1] nonan-	Flower	Not Found
9,12-Octadecadienoic Acid	Flower	Not Found
Acteamine, N-(1,1,3,3-tetramethylbutyl)-	Flower	Organic synthesis and chemical medicine [69]
Acetophenone	Flower	Antifungal [70] and flavoring agents [71]
Alpha Amyrin	Leaf	Anti-inflammatory, anxiolytic, and anti-depressant [72]
Hentriacontane	Flower	Anti-inflammatory, anti-tumor, and Antibacterial [73]
Hexadecane, 2,6,10,14-Tetramethyl-	Flower	Not Found
Icosane	Flower	It has thermal properties [74]
Nonacosane	Flower	The main paraffin of most plant waxes [74]
Nonadecane	Flower	Not Found
Octacosane	Leaf	Antioxidant [75]
Stigmast-5-EN-3-OL	Leaf and Flower	Anti-diabetic, cholesterol reduction and anti-cancer [76]
Stigmasterol	Leaf and Flower	Immune response, increasing the concentration of enzymes [77], and -anti-cancer [78]
Vitamin E	Leaf and Flower	Chronic disease prevention, antioxidants, and neurological therapy [79]
Z,Z-11,13-Hexadecadien-1-ol	Flower	Medical, pharmaceutical, and cosmetic components [80]

CONCLUSION:

This study was one of the few reports in which the bioactive compound extracts of *H. longiflora* (L.) G. Don were reported. Various bioactive compounds were found in *H. longiflora* (L.) G. Don. Thirty-seven chemical constituents of *H. longiflora* (L.) G. Don leaf and flower extracts were identified with GC-MS. Squalene, 2,4',6- Trimethyldiphenylsulfone and Vitamin E which predominate in the leaves act as a producer of high amounts of antioxidants and also as a medium for cancer treatment. Ellipticine, Vitamin E, and hentriacontane predominate in flowers with their role as anti-neoplastic agents that can prevent, kill, or inhibit the growth and spread of cancer cells. Squalene and other bioactive compounds highlight the use of drugs and pharmaceuticals. Based on phytocomponents identified with potential biological activity, this plant can be an essential ingredient in medicine. Its ability as an anti-tumor, anti-cancer, antibacterial, and antioxidant drug from this plant as a natural constituent source can be explored further.

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COMPETING INTERESTS:

The authors declare that they have no competing interests.

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