Nano-Phytosome Drug Delivery System of Acanthus Illicifolius Leaves Extract: Characterization, Formulation and Evaluation

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Abstract

Achantus Illicifolius plants are very rarely exploited but many are found to grow wildly in nature. If the conditions of the aquatic environment in Indonesia especially in Purworejo today, cranberry plant has potential as a phytotechnology. Phytosomes are a novel lipid-based delivery system that resemble liposomes in structure. They can be used to entrap various phytoconstituents based on polyphenols to enhance their absorption when administered. The purpose of this study was to investigate the potential compounds of Acanthus illicifolius, jeruju extract containing nanophytosome formulation and physicochemical characterization. The test material was Achantus ilicifolius leaf obtained from the Purworejo Mangrove Conservation Center, Central Java, Indonesia. General tests for the presence of the following; flavonoid, Saponin, Terpenoid and Steroid were carried out. The bioactive compounds in the methanol extract of the leaves of Achantus Ilicifolius were identified using GC-MS. Nano-phytosomes were formulated by making three variations in the ratio of phosphatidylcholine starting from 24 mg (F1) : 48 mg (F2) : 71 mg (F3) with using the reflux method. Evaluation of nano-phytosomes includes particle size and zeta potential. The evaluation results show that the nano-phytosome particle size is around 122.7 nm – 193.5 nm. The nano-phytosome formula with the best adsorption efficiency is F3 with a weight ratio of phosphatidylcholine 71 mg, a particle size replication 1 of 120.7 nm, replication 2 of 121.6 nm, replication 3 of 125.8 nm and zeta potential -26,6.

Keywords: Nano-Phytosome; Acanthus Illicifolius; Formulation; Evaluation; Leaves Extract; Drug Delivery.

Introduction

Mangrove plants in Indonesia are the largest in the world, both in terms of area quantity (+42,550 km2) and number of species (+ 45 species) [1]. West Sumatra Province, which is located on the seashore, is also rich in mangrove plants. Mangroves have many benefits that are directly related to human life, ranging from ecological benefits to being a source of food and medicine. Many universities and research institutions have explored various plants as sources of medicine but have not looked much into the properties of mangrove plants [1], [2]. The plant Acanthus ilicifolius contains bioactive compounds that exhibit a range of potential health benefits, including antimicrobial, anticancer, antiallergic, anthelmintic, anti-inflammatory, antioxidant, antileishmanial, osteoblastic, and hepatoprotective properties [3], [4], [5]. It is used in traditional medicine to treat skin diseases, helminthiasis, allergies, dyspepsia, paralysis, headaches, asthma, and rheumatism [3], [6]. A. ilicifolius is utilized as a phytoremediation agent for copper in Indonesia [7]. Achantus Illicifolius plants are very rarely exploited but many are found to grow wildly in nature. If the conditions of the aquatic environment in Indonesia especially in Purworejo today, cranberry plant has potential as a phytotechnology.

Phytosomes are a novel lipid-based delivery system that resemble liposomes in structure. They can be used to entrap various phytoconstituents based on polyphenols to enhance their absorption when administered [8], [9], [10]. Poorly soluble polyphenolic compounds can be significantly improved in terms of penetration and absorption across the biological membrane as well as bioavailability by being encapsulated into the phytosomal delivery system [11]. The majority of phytochemicals with biological activity found in plants that can be applied in the pharmaceutical and cosmeceutical industries have chemical structures based on polyphenolic compounds [12], which possess high molecular weight, a multiple-ring structure, and high miscibility in the aqueous phase [13], [14]. Because of their distinct physicochemical characteristics, nanosized drug delivery systems can be used to improve the penetration of bioactive polyphenolic phytocompounds across biological barriers, thereby increasing their bioavailability and mitigating their low absorption [15], [16], [17].

Research on the bioactive compounds found in Indonesian Acanthus illicifolius, especially those found in Purworejo waters, is still lacking. Therefore, research related to the phytochemical contents of Acanthus ilicifolius and its potential in phytotechnology should be carried out. The information generated is expected to enhance the scientific reach, strengthen the conservation efforts of species and serve as a basis for the development of research on medicinal plants and technology of drug delivery systems. The purpose of this study was to investigate the potential compounds of Acanthus illicifolius as sources of pharmacological activity.

Material and methods

Tools

The tools used in the research include grinding, rotary evaporator, freeze drying, thin layer chromatography plates, and glassware.

Ingredients

The test material was Achantus ilicifolius leaf obtained from the Purworejo Mangrove Conservation Center, Central Java, Indonesia. The chemicals used are 96% methanol, 2 N sulfuric acid, ethanol, n-hexane, ethyl acetate, formic acid, toluene, ethyl acetate, Lieberman Bucard reagent, chloroform, sulfuric acid anisaldehyde reagent, nitrogen.

Preparation of Acanthus ilicifolius L . Leaf Extract

Achantus ilicifolius leaves weighing as much as 1.000 g were washed, cut, and dried. After drying, it was pollinated using an 80 mesh sieve to become simplicia powder. The leaf powder of Achantus ilicifolius was then extracted by the maceration method in 96% methanol at a ratio of 1:3 for eight days to obtain a filtrate that no longer contained the active substance. The filtered macerates were collected and evaporated with a rotary evaporator at a temperature of 48°C and a speed of 90 rpm until all the methanol evaporated to make a thick extract. The extract was then freeze-dried to remove the water content.

Preliminary phytochemical screening TLC-densitometry

General tests for the presence of the following; flavonoid, Saponin, Terpenoid and Steroid were carried out following methods previously described:

a. Flavonoid Qualitative Test

   Take samples carefully. Add 1 mL of ethanol. Vortex and sonicate for 60 minutes. Maceration for 24 hours. Spot a sample of 20 µl on a silica gel plate, including a routine standard. Insert the plate into the saturated chamber of the mobile phase n-hexane: ethyl acetate: formic acid (6:4:0,1). Expand to the limit. Dry the plate. Observe under UV light.

b. Steroid Qualitative Test

   Take the sample carefully. Add 2 mL of ethanol, vortex for 2 minutes. Centrifuge for 3 minutes. Apply 20 µl to the 60 F254 silicagel plate. Include β-sitosterol standard. Enter into the saturated chamber the mobile phase Toluene: Ethyl Acetate (80:20). Expand to the limit, remove and dry. Spray with Lieberman Bucard reagent. Heat at 110°C for 2 minutes.

c. Saponin Test

   Take 100 mg sample. Put into the flask, add 10 mL of 2 N sulfuric acid. Hydrolysis/reflux with reverse cooling for 30 minutes. Cool and extract with 5 ml of chloroform. Take chloroform phase, vaporize with nitrogen. Add up to 500 µL. Apply 10 µL to the 60 F254 silicagel plate. Enter the chamber containing the saturated mobile phase chloroform: methanol (95:5), expand to the limit, remove the plate and dry. Observe under UV light, spray with sulfuric acid anisaldehyde reagent, heat at 110°C to maximum spot.

d. Terpenoid qualitative test

Take a sample of 0.2 mL. Add 2mL of n-hexane, vortex for 2 minutes. Centrifuge for 3 minutes. Spot the sample as much as 10 l on 60 F254 silicagel plate. Include terpeniol standard. Enter into the saturated chamber the mobile phase toluene – ethyl acetate (93:7). Expand to the limit, remove and dry. Spray with vanillin sulfuric acid reagent. Heat at 110ºC for 2 minutes.

GC-MS analysis

The bioactive compounds in the methanol extract of the leaves of Achantus Ilicifolius were identified using GC-MS. The GC-MS analysis method is through reading the spectra on GC and MS. A sample of 1µL was injected into GC-MS which was operated using an HP-5MS UI glass column 30 m long, 0.25 mm in diameter and 0.25 m thick with a carrier gas, namely Helium UHP (He). The column temperature used is 50ºC and the injection temperature is 260ºC. The GC chromatogram profile and MS spectra showed the number of compounds that could be identified. Determination of compounds based on similarity index and compound fragmentation pattern [18].

Table 1. Column temp

No	Retention Time (min)	Rate (ºC/min)	Target value (ºC)	Hold time (min)
1	2	0	50	2
2	60.0	5	240	20

Formulation and Evaluation

The lyofilization powder is weighed at 5 mg for all formulas, then dissolved in 10 mL of ethanol p.a. The cholesterol used in this study was very small, only 0.3249 mg. The weight could not be weighed using an analytical balance because the minimum weighable weight was 1 mg so that the cholesterol was made in the form of a stock solution. A total of 12.5 mg of cholesterol is dissolved with chloromethane in a 25 mL pumpkin. The concentration of such a cholesterol solution is 0.5 mg/ml. The lyofilized powder and phosphatidylcholine that are already in dissolved state are then mixed in a round pumpkin. The drug was rinse using 2 mL of 96% ethanol p.a. Added a 0.5 mg/mL cholesterol solution to a 650 µL round pumpkin. After the reflux process is completed, the solution is poured into a petri cup. The solvent is applied with a hair dryer for ± 30 minutes until a thin layer of phytosome is formed on the surface of the petri Cup. Hydrating the thin layer of the phytosome with 20 mL of aquabidest using a magnetic stirrer for 30 minutes. Then the solution is transferred into a 20 mL vial and the particle size is reduced using a 30 minute ultrasonic process. The preparation is then characterized by measuring the size of the particle and the zeta potential. Particle size was analyzed with Horiba SZ-100 using Dynamic Light Scattering (DLS) technique. Particle surface shapes were analyzed using SEM (Sanning Electron Microscopy) testing [19].

Table 2: Nano-Phytosome formulation

Materials	F1	F2	F3
Lyophilized powder of Acanthus Illicifolius Leaves Extract (mg)	10	10	10
Phosphatidylcholine (mg)	24	48	71
Cholesterol (mg)	0.3249	0.3249	0.3249
Dichloromethane p.a (mL)	5	5	5
Ethanol p.a (mL)	20	20	20
Aquabidest (mL)	25	25	25

Results and Discussion

Flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids were all present in the methanolic extract of A. ilicifolius used in this investigation (Table 3).

Table 3: Phytochemical detected in a methanolic extract of Acanthus illicifolius leaves

Metabolites	Results
Flavonoids	+
Steroids	+
Saponins	+
Terpenoids	+

Note: (+) = presence, (-) = absence

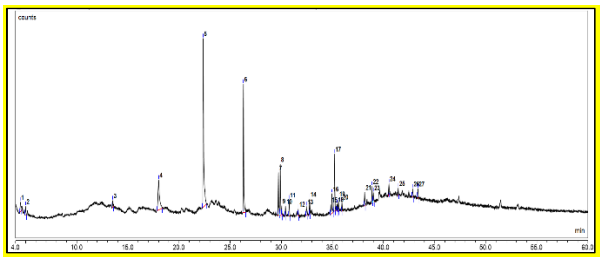


Figure 1: Chromatogram of 70% methanol extract of Acanthus Ilicifolius Leaves

This outcome is comparable to that of Gayathri and Gayathri (2014), who discovered the presence of flavonoids, glycosides, phenols, tannins, steroids, saponins, and terpenoids in methanolic extracts of A. ilicifolius leaves [20]. According to Andriani (2020), A. ilicifolius leaves methanolic extracts included alkaloids, flavonoid, glycosides, polyphenols, steroids, tannins [21]. Alkaloids, flavonoids, glycosides, and tannins were identified in the methanolic extracts of A. ilicifolius by Poorna (2011) [22].

Flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids were all present in the methanolic extract of A. ilicifolius used in this investigation (Table 3).

Table 4: Results of identification of GC-MS 70% methanol extract

No.	Ret.Time min	Hit# 1	Chemical Formula	Mol. Weight	SI Hit#1	Rel.Area (%)
1	4,46	Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]	C12H19FN2O	226	639	1.93
2	4,98	12,15-Octadecadiynoic acid, methyl ester	C19H30O2	290	671	1.00
3	13,51	3,6,9,12-Tetraoxatetradecan-1-ol, 14-[4-(1,1,3,3-tetramethylbutyl)phenoxy]	C24H42O6	426	585	0.97
4	18,00	Cyclohexasiloxane, dodecamethyl	C12H36O6Si6	444	735	10.79
5	22,36	Cycloheptasiloxane, tetradecamethyl	C14H42O7Si7	518	767	28.23
6	26,30	Cyclooctasiloxane, hexadecamethyl	C16H48O8Si8	592	848	15.51
7	29,72	Cyclononasiloxane, octadecamethyl	C18H54O9Si9	666	761	4.91
8	29,90	Ethanol, 2-(9-octadecenyloxy), (Z)	C20H40O2	312	775	5.60
9	30,04	Ethyl iso-allocholate	C26H44O5	436	708	0.81
10	30,41	Ethyl iso-allocholate	C26H44O5	436	713	0.97
11	30,76	13-Heptadecyn-1-ol	C17H32O	252	724	1.77
12	31,66	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	C25H42O2	374	727	0.68
13	32,46	Ethyl iso-allocholate	C26H44O5	436	726	1.49
14	32,77	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	C16H50O7Si8	578	700	1.70
15	34,84	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)	C21H36O4	352	721	1.11
16	34,96	11-Octadecenoic acid, methyl ester	C19H36O2	296	752	2.85
17	35,20	Phytol	C20H40O	296	790	6.77
18	35,44	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	C25H42O2	374	731	0.75
19	35,59	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	C16H50O7Si8	578	664	1.34
20	35,92	Ethyl iso-allocholate	C26H44O5	436	726	1.06
21	38,15	Ethyl iso-allocholate	C26H44O5	436	669	1.74
22	38,89	Ethyl iso-allocholate	C26H44O5	436	749	1.83
23	39,00	Ethyl iso-allocholate	C26H44O5	436	744	1.47
24	40,52	Ethyl iso-allocholate	C26H44O5	436	692	1.50
25	41,44	Ethyl iso-allocholate	C26H44O5	436	751	0.84
26	42,84	Ethyl iso-allocholate	C26H44O5	436	726	1.19
27	43,37	Ethyl iso-allocholate	C26H44O5	436	695	1.18

Flavonoids, alkaloids, glycosides, polyphenols, tannins, and steroids were all present in the methanolic extract of A. ilicifolius used in this investigation (Table 3). This outcome is comparable to that of Gayathri and Gayathri (2014), who discovered the presence of flavonoids, glycosides, phenols, tannins, steroids, saponins, and terpenoids in methanolic extracts of A. ilicifolius leaves [20]. According to Andriani (2020), A. ilicifolius leaves methanolic extracts included alkaloids, flavonoid, glycosides, polyphenols, steroids, tannins [21]. Alkaloids, flavonoids, glycosides, and tannins were identified in the methanolic extracts of A. ilicifolius by Poorna (2011) [22].

The primary phytochemical components of the shoot were successfully identified by GC-MS analysis (Table 4). Sixteen major peaks, respectively, were revealed by GC-MS analysis of an extract of Acanthus illicifolius leaves dissolved in methanol solvents. These peaks indicated the presence of different phytochemical constituents. Through a comparison of the constituents' mass spectra with the main library, each of these compounds was characterized and most likely identified. In the control shoot's methanol extract, five different compounds were found. Cyclohexasiloxane-dodecamethyl (10.79%), Cycloheptasiloxane-tetradecamethyl (28.23%), Cyclooctasiloxane-hexadecamethyl (15.51%), Cyclononasiloxane-octadecamethyl (4.91%), Ethanol, 2-(9-octadecenyloxy)-(Z) (5.60%), Phytol (6.77%) were the five compounds with the highest relative abundance. 28.19 percent of the extract was made up of other substances.

Cyclohexasiloxane, dodecamethyl; hexadecanoic acid, methyl ester which are responsible for anti-inflammatory and analgesic activity [23]. Phytol (PYT), an essential oil derived from diterpenes and a component of chlorophyll, is found in abundance in nature [24]. It is a fragrant component that is primarily present in cosmetics, toilet soaps, shampoos, and other toiletries. Occasionally, non-cosmetic items like detergents and household cleansers contain it as well [25]. PYT and a number of its derivatives have recently been shown to have distinct pharmacologic functions in both humans and other animals [26], [27], [28], [29], [30]. Pharma-medico perspectives have demonstrated the antimicrobial, cytotoxic, antitumorous, antimutagenic, anti-teratogenic, antibiotic-chemotherapeutic, antidiabetic, lipid-lowering, antispasmodic, anticonvulsant, antioxidant, anti-inflammatory, anxiolytic, antidepressant, immunoadjuvant, hair growth facilitator, hair fall defense, and antidandruff properties of PYT and its derivatives [31].

On Table 4 can be concluded all formulas have sizes that meet the requirements as nanoparticles. The diameter of the formula 3 (F3) particle size is the smallest of the three formulas so that the active substance can be absorbed more optimally. On particle size distribution testing it is known that the three formulas fall into the category of particle nanometers where the distributed particle sizes are smaller than 200 nm.

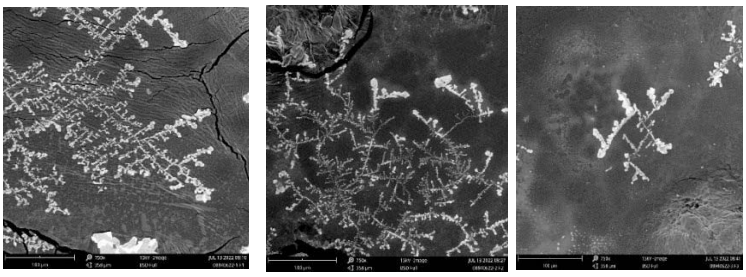
Table 5: Particle size and zeta potential

Replication	Particle size (nm) F1	Particle size (nm) F2	Particle size (nm) F3
1	194	173.9	120.7
2	193	173.3	121.6
3	193.5	176.8	125.8
Zeta Potential (mV)	-15.1	-25.9	-26.6

Nanoparticles are 1-1000 nm particles but many suggest preferably particle diameter sizes of 200-400 nm [32]. The size of human cells is 10,000 to 20,000 nm in diameter, and large biological macromolecular organels like hemoglobin are 5 nm diameter and the bilayer lipid cells surrounding them are 6 nm thick, so it takes nanoparticles smaller than 20 nm to penetrate the walls of the blood vessels. As a drug carrier it is recommended to have nanoparticle sizes ranging from 200 nm to avoid rapid filtration by the lymph. However, to reach the liver, the drug particles must be 150-200 nm [33]. In addition, the potential zeta value of the three formulas has a negative charge. For the third stability the formula tends to be stable especially in Formula 3 has a good stability as it approaches the value (+/-) 30. High zeta potential values can prevent the occurrence of aggregation of the particle because there is a rejection of the force between the particles and the presence of

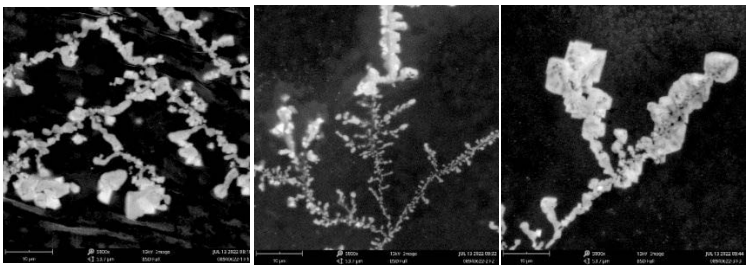
electrically stability of the dispersion of the nanoparticles. In particles with a small potential zeta value indicates a greater attraction-attraction force than the rejective force resulting in coagulation and flocculation [34]. Nanoparticles with a zeta potential value (+/-) 30 mV have a high stability. A dispersed system with a low potential zeta value is easier to form an aggregate with a Van der Waals style in the interaction of particles [32].

SEM tests are performed to determine the morphology of the particles of the nano-phytosome preparation. The way the scanning electron microscope (SEM) works is different from the way the optical microscopes use light rays while the SEM uses electron rays that are then fired towards the specimen to produce an image of the surface of the specimens [35]. This time using an electron signal which can produce a topography of the specimen, on a surface that has a brighter color indicates that the surface is higher than a darker surface.



F1 **F2** **F3**

Figure 2: Morphology of nano-phytosome preparations at 750x magnification with an acceleration voltage of 15kV



F1 **F2** **F3**

Figure 3: Morphology of nano-phytosome preparations at 5,000x magnification with an acceleration voltage of 15kV

In Figure 2 and 3 it is known that the particles present in the three formulas are strongly bound to each other so that they form a kind of enveloping layer outside the particle so that it can be said that the extracts of the leaf lifolysis powder are wrapped with the formula of a phytosome at a nano-sized size which corresponds to the concept of a nanophytosome. The results of the Scanning Electron Microscopy (SEM) show that from the total production of nanoparticles, it can be seen that some of the nanoparticle produced is already nano-sized. Electron Microscopy Scanning (SEM) is a method to study the surface structure of samples. This instrument gives a large field depth, that is, the area of the sample can be seen in a fairly large focus. SEM has the advantage of a relatively wide zoom range. The images produced by this instrument appear in a three-dimensional form, making it more attractive to the human eye and making it easier for researchers to analyze. Scanning Electron Microscopy (SEM) provides surface information that details information by tracking samples in raster patterns with electron files. The spread pattern created by the samples interaction with the electron files generates information about the size, shape, texture and composition of samples. [36], [37]. In Table 4 it is shown that nanoparticles F1, F2 and F3 are in sequence in the range of 122.7 nm – 193.5 nm, the nanoparticle size required in the drug delivery system is 50-300 nm [38]. The size of the nanoparticles F1, F2 and F3 on the life-polytic powder extract meets the range of nanoparticle size in the drug delivery system. This is in line with the research carried out by (Sabdoningrum et al, 2021) that the synthesis of nanoparticles extracted from dredging (Phyllanthus niruri Linn) by the method of ball milling produces an average nanoparticle size of 192.6 nm with the result of the SEM nanoparticule extract of dredding (Phylanthus Niruri Lin) shows amorphous morphology and the presence of agglomeration in milling processes that have small particle size morphologies of size [39]. The research conducted by (Husni & Puspitaningrum, 2017) has an average size of nanophytosome particle diameter in the range of 52.1 – 101.9 nm, the diameter has a smaller range compared to the study currently conducted by us. The results show that the average diameter of nano-phytosomal particle made in accordance with the average particle size of diameter resulting from complexion between active ingredients in plants with phosphatidylcholine is in the area of 50 nm – 500 μm [40]. Based on research carried out by (Nazemoroaya et al, 2022) has a saponinosome size of about 60 nm and a zeta potential of about -30 mV. SEM and AFM analysis provides information on the morphology of the saponinosome. The results show that the saponinosome has a round shape with a smooth surface, and little agglomeration is observed [41]. While (Nandhini & Ilango, 2021) has a surface morphology of an optimized phytosome showing a round-shaped phytoscopic vesicle with an average diameter of 226.22 ± 31.97 nm, the diameter is larger than the results of the current study. Phytosome-sized distribution particles are uniform that indicate the absence of phospholipid aggregation [42].

Conclusion

The bioactive compounds in the methanol extract of the leaves of Achantus Ilicifolius were identified using GC-MS. Cyclohexasiloxane-dodecamethyl (10.79%), Cycloheptasiloxane-tetradecamethyl (28.23%), Cyclooctasiloxane-hexadecamethyl (15.51%), Cyclononasiloxane-octadecamethyl (4.91%), Ethanol, 2-(9-octadecenyloxy)-(Z) (5.60%), Phytol (6.77%) were the five compounds with the highest relative

abundance. The nano-phytosome particle size is around 122.7 nm – 193.5 nm that nanoparticle size required in the drug delivery system. The nano-phytosome formula with the best adsorption efficiency is F3 with a weight ratio of phosphatidylcholine 71 mg, a particle size replication 1 of 120.7 nm, replication 2 of 121.6 nm, replication 3 of 125.8 nm and zeta potential -26,6 have a high stability.

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Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

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