Mircowave-Assisted Extraction of Phytochemical Constituents in Roselle (Hibiscus sabdariffa L.)

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Author’s contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Roselle (Hibiscus sabdariffa L.) is natural source of valuable components such as phenolics, flavonoids and anthocyanins. Anthocyanin is normally used in the food technology to replace synthetic pigments as well as medicinal properties due to antioxidants. Microwave irradiation is a feasible green solvent extraction method receiving great attention as it utilizes solvent at elevated temperature and controlled pressure conditions. Microwave extraction emerges as a promising inexpensive, simple and efficient technique. In our research, various variable like microwave power (20, 40, 60, 80, 100 W), microwave frequency (10, 20, 30, 40, 50 GHz), the microwave irradiation duration (5, 10, 15, 20, 25 min), liquid to solid ratio (water: material, 2:1, 3:1, 4:1, 5:1, 6:1), extraction temperature (50, 55, 60, 65, 70°C), number of extraction cycles (1, 2, 3, 4, 5) influencing to the microwave-assisted extraction of phytochemicals in roselle (Hibiscus sabdariffa L.) calyx were thoroughly examined. Total total phenolic (mg GAE/ 100 g), total flavonoid (mg GE/ 100 g), anthocyanin (mg/100 g) were key indicators to define the optimal variable. Our results revealed that microwave power at 80 W, frequency 40 GHz, duration 15 min, liquid to solid ratio 4:1, temperature 55°C, 4 cycles of extraction were appropriate for extraction of phytochemical components inside roselle (Hibiscus sabdariffa L.) calyx.

Keywords: Roselle calyx; Hibiscus sabdariffa L.; microwave extraction; phenolic; flavonoid; anthocyanin.
1. INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is an ideal crop for developing countries. Rain or high humidity during the harvest time and drying process can downgrade the quality of the calyces and reduce the yield. It can be used as food and fibre [1]. Roselle contains protein, fat, carbohydrate, fiber, and mineral, vitamin. The chemical components contained in *Hibiscus sabdariffa* calyx include anthocyanins, flavonoids, polyphenols polysaccharides and organic acids having different modern therapeutic applications [2-6]. Phenolic derivatives and flavonoids natural compounds in plants and play several roles in the plant’s life such as general growth, reproduction, and defence against parasites and pests [7]. Flavonoids themselves are a group of hydroxylated phenolic compounds having a benzo-γ-pyrene structure and are ubiquitously occurring in plants [8]. Anthocyanins are steroid or triterpenoid glycosides, common in a large number of herbs. According to the structure of the aglycone or sapogenin, anthocyanins are classified as neutral and acid type, the so-called neutral anthocyanins are derivatives of steroids with spiroketal side chains which are almost exclusively present in the monocotyledonous angiosperms and the acid anthocyanins that possess triterpenoid structure type, which is the most common and occur mainly in the dicotyledonous angiosperms. It has effect on cold blooded animals, also to have the analgesic, anti-nociceptive, antioxidant activity, to impair the digestion of protein, to cause hypoglycemia and to act as anti-fungal and antiviral agents [9]. The amount of L-ascorbic acid extracted was 83.1 mg/100 g [10]. *Hibiscus sabdariffa* L. calyx contained polyphenols of the flavonol and flavanone type in simple or polymerised form [11].

Extracts of dried calyces were able to decrease low-density lipoprotein cholesterol, triglyceride, total cholesterol and lipid peroxidation [12-14]. According to one report, the total phenolic content was found to be 41.07 mg GAE/g [15]. According to another research, roselle had total phenols 29.178 mg/kg [16]. Meanwhile, flavonoid was observed in calyx extracts 148.35 mg/g [17]. Fresh or dried calyces of *Hibiscus sabdariffa* L. are utilized to convert into herbal tea, hot and cold beverage, fermented drink, soft drink, wine, jam, jellied confectionary, sauce, marmalade, ice cream, chocolate, flavouring agent, pudding and cake [18-26]. Aqueous extracts of roselle (*Hibiscus sabdariffa* L.) calyces have characteristic intense red colouration due to the presence of anthocyanins which could be utilised as colouring agent in pharmaceutical products [27,28]. It has been utilized to cure different degenerative diseases like hypertension, hyperlipidemia, cancer and other inflammatory diseases of liver and kidney [29]. It’s also proven to treat cardiac, diuretic, sore throat and cough, cholerectic, febrifugal and hypotensive effect, liver disorder, decrease the viscosity of the blood, induce lactation and stimulate intestinal peristalsis [30-33].

Microwave-extracted extraction is a method that utilizes a solvent to extract the phytochemical components from herbs. In this process, the improved extraction happens as a result of change in the herbal cell texture created by electromagnetic waves [34]. Microwaves are electromagnetic radiations having frequency from 0.3 to 300 GHz. The combination of thermal and mass gradients contribute to the high yield and short extraction duration of micro-extracted extraction. The variables that affect the extraction recovery of this extraction process are the power and frequency of the microwaves, the duration of the microwave irradiation, the moisture content and particle sizes of the herbs, the kind and concentration of the solvent, the ratio of solid to liquid, the extraction temperature, the extraction pressure, and the number of extraction cycles [35]. Microwave-assisted extraction (MAE) has been recognized as a technique with several advantages over other extraction methods, such as reduction of costs, extraction time, energy consumption, and CO₂ emissions. However, there was not many research mentioned to the application of microwave-assisted extraction to extract phytochemicals in roselle. Maceration and ultrasound-assisted techniques were compared in assessing the extraction performance of anthocyanin in roselle [36]. Objective of our study focused on various variables such as microwave power, microwave frequency, the microwave irradiation duration, liquid to solid ratio, extraction temperature, number of extraction cycles affecting to the microwave-assisted extraction of phytochemicals in roselle (*Hibiscus sabdariffa* L.) calyx.

2. MATERIALS AND METHODS

2.1 Materials

Roselle (*Hibiscus sabdariffa* L.) calyxs were naturally collected from Hau Giang province, Vietnam. After collecting, they must be kept in dry cool box and quickly conveyed to laboratory for experiments. They were subjected to washing
and treatment. These calyxs were treated by different parameters such as microwave power (20, 40, 60, 80, 100 W), microwave frequency (10, 20, 30, 40, 50 GHz), the microwave irradiation duration (5, 10, 15, 20, 25 min), liquid to solid ratio (water: material, 2:1, 3:1, 4:1, 5:1, 6:1), extraction temperature (50, 55, 60, 65, 70°C), number of extraction cycles (1, 2, 3, 4, 5). At the end each treatment, samples were analyzed total phenolic (mg GAE/100 g), flavonoid (mg GE/100 g), anthocyanin (mg/100 g) content to define the optimal value.

2.2 Researching Methods

2.2.1 Effect of microwave power in phytochemical extraction of Hibiscus sabdariffa L. calyx

Raw Hibiscus sabdariffa L. calyx was extracted by various microwave power values (20, 40, 60, 80, 100 W). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were key indicators so they were chosen to define the optimal microwave power.

2.2.2 Effect of microwave frequency in phytochemical extraction of Hibiscus sabdariffa L. calyx

Raw Hibiscus sabdariffa L. calyx was extracted by different microwave frequency values (10, 20, 30, 40, 50 GHz). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were major indicators so they were chosen to determine the optimal microwave frequency.

2.2.3 Effect of microwave irradiation duration in phytochemical extraction of Hibiscus sabdariffa L. calyx

Raw Hibiscus sabdariffa L. calyx was extracted by different microwave irradiation duration values (5, 10, 15, 20, 25 min). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were main indicators so they were chosen to select the optimal microwave irradiation duration.

2.2.4 Effect of liquid to solid ratio (water: Hibiscus sabdariffa L. calyx) in phytochemical extraction

Raw Hibiscus sabdariffa L. calyx was extracted by different liquid to solid ratio (water: Hibiscus sabdariffa L. calyx, 2:1, 3:1, 4:1, 5:1, 6:1). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were key indicators so they were chosen to estimate the optimal liquid to solid ratio.

2.2.5 Effect of extraction temperature (°C) in phytochemical extraction of Hibiscus sabdariffa L. calyx

Raw Hibiscus sabdariffa L. calyx was extracted by different extraction temperature values (50, 55, 60, 65, 70°C). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were major indicators so they were chosen to identify the optimal extraction temperature.

2.2.6 Effect of the number of extraction cycles in phytochemical extraction of Hibiscus sabdariffa L. calyx

Raw Hibiscus sabdariffa L. calyx was extracted by different numbers of extraction cycles (1, 2, 3, 4, 5). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were important indicators so they were chosen to identify the optimal number of extraction cycles.

2.3 Chemical and Statistical Analysis

Total polyphenol content (mg GAE/100 g) was determined by FolinCiocalteu reagent method [37]. Aluminum chloride colorimetric method was used for flavonoids (mg QE/100 g) determination [38]. Total anthocyanin content (mg) was quantified by spectrophotometry [39]. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Statgraphics Centurion XVI.

3. RESULTS AND DISCUSSION

3.1 Effect of Microwave Power (W) in Phytochemical Extraction of Hibiscus sabdariffa L. Calyx

Extract of roselle calyces has shown the presence of biochemicals such as anthocyanins, protocatechuic acid, flavonoids, and anthocyanin [12,40-43]. According to one report, Roselle calyces contained ascorbic acid (140.13 mg/100 g), total anthocyanins (622.91 mg/100 g) and total phenolics (37.42 mg/g) [44]. Meanwhile the anthocyanin was detected at 1.8% in red roselle calyx [45]. Polyphenols in roselle included delphinidin and cyanidin having antioxidant activities beneficial for human body [46-49].
Aqueous extracts of roselle (Hibiscus sabdariffa L.) calyces have characteristic intense red colouration due to the presence of anthocyanins, which are flavonoids are water-soluble natural pigments [50]. In our research, raw Hibiscus sabdariffa L. calyx was extracted by different microwave power values (20, 40, 60, 80, 100 W). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100g) were important indicators so they were chosen to identify the optimal microwave power. Our result showed that when microwave power increased from 20 W to 80 W, the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx increased. There was no significant difference at microwave power 80 W and 100 W so the optimal microwave power should be 80 W to maintain the highest content of total phenolic, flavonoid and anthocyanin. In one report, variable of power had no important effects on the yield of flavonoids [51]. One studied microwave extraction of cardamom. When the glands were subjected to more severe thermal stresses and localized high pressures, pressure build-up happens within the glands which results in accelerated capacity for expansion and leading to cell rupture more rapidly than in traditional extraction [52].

3.2 Effect of Microwave Frequency (GHz) in Phytochemical Extraction of Hibiscus sabdariffa L. Calyx

Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz or between wavelengths of 1 cm and 1m [53]. Microwave energy is transferred directly to the herbal tissue through molecular interaction the electromagnetic field via conversions of electromagnetic energy into thermal energy [35,54]. In our research, raw Hibiscus sabdariffa L. calyx was extracted by different microwave frequency values (10, 20, 30, 40, 50 GHz). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100g) were main indicators so they were chosen to define the optimal microwave frequency. Our result showed that when microwave frequency increased from 10 GHz to 40 GHz, the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx increased also. There was no significant difference at microwave frequency 40 GHz and 50 GHz so the optimal microwave frequency should be conducted at 40 GHz to preserve the highest content of total phenolic, flavonoid and anthocyanin.

3.3 Effect of Microwave Irradiation Duration (min) in Phytochemical Extraction of Hibiscus sabdariffa L. Calyx

The microwave power and irradiation times influence each other to a great extent [53]. In our research, raw Hibiscus sabdariffa L. calyx was extracted by different microwave irradiation duration values (5, 10, 15, 20, 25 min). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were key variables to identify the optimal microwave irradiation duration. Our result showed that when microwave irradiation duration increased from 5 minutes to 15 minutes, the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx increased also. However if microwave irradiation duration extended over 15 minutes, these phytochemical constituents decreased gradually. So the optimal irradiation duration should be 15 min to extract the highest content of total phenolic, flavonoid and anthocyanin. Total phenolic content was extracted from aromatic plants such as Rosmarinus officinalis using microwave-assisted extraction. Compared with traditional reflux extraction, microwave-assisted extraction reduced extraction duration, limited solvent volume and increased extraction yield of total phenolics [55]. The yield of analyte extracted can be enhanced with an accelerate in the extraction time; however, there is a related risk of degradation of sensitive components [56]. One study confirmed that extraction duration in microwave assisted process was found to decrease with increase in temperature. This decrease could be realized to the fact that with increase in temperature, the vapour pressure of water present inside the celery seeds increased leading to leaching out and evaporation of volatile oil along with moisture [57]. Extended extraction durations increased the yield of total flavonoid, while progressively decreased flavonoids and antiradical power [58].

3.4 Effect of Ratio of Liquid to Solid (Water: Hibiscus sabdariffa L. Calyx) in Phytochemical Extraction

Another critical factor in microwave-assisted extraction is the ratio of the solid herbs to the amount of solvent. The bioactive ingredients in the herbs can effectively dissolve when large amounts of solvent are used, thereby leading to improved extraction yields. Solvent specification
is the most vital variable because the solvent affects the absorbance of the microwave energy, as determined by the dissipation factor [59,60]. The solvent must have an affinity for the target component and an capability to absorb microwave energy [61]. In a higher contact surface area, the extraction efficiency rises. Similarly, finer particles permit enhanced penetration of the microwave. Fine particles may stance some technical difficulties; filtration is applied to prepare the matrix. In our research, raw Hibiscus sabdariffa L. calyx was extracted by different liquid to solid ratios (water: Hibiscus sabdariffa L. calyx, 2:1, 3:1, 4:1, 5:1, 6:1). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were major variables to identify the optimal liquid to solid ratio. Our result showed that when liquid to solid ratio increased, the phytochemical contents decreased afterward. The optimal liquid to solid ratio should be 4:1 to receive the highest content of total phenolic, flavonoid and anthocyanin. In another report, the effect of solvent types and solute to solvent ratios were also evaluated by maceration and ultrasound-assisted to extract anthocyanin. Water was a better choice of solvent as compared to ethanol [36]. The optimum conditions for fresh roselle calyxes, fresh calyxes to water ratio was 1:2. For dried roselle calyxes, the optimum conditions were 1:10 ratio of dried calyxes to water. The total anthocyanin, total phenolic contents in fresh roselle calyxes were 37.67±0.02 mg/100 g, 31.26±0.75 mg gallic acid/g. The total anthocyanin, total phenolic contents in dried roselle calyxes were 340.97±0.15 mg/100 g, 31.18±0.62 mg gallic acid/g [62]. Anthocyanin from roselle has been previously extracted using various solvents such as water [63-68], methanol [63], ethanol acidified by HCl [69] and in the instant pressure drop system solvent [70]. A high solvent ratio with microwave-assisted extraction showed consistent results [71,72]. When large quantities of solvent were utilized, the extraction yield initially increased and then decreased as the solid-to-liquid ratio decreased [72,73]. When the amount of solvent was reduced (high-solid herbal materials), microwave energy may have been absorbed and dispersed by the large amount of plant materials [71], thereby increasing the solid mass, and decreasing the surface area available for solvent to penetrate the plant materials and solubilize the target molecules [74].

Table 1. Effect of microwave power to the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx

| Microwave power (W) | 20  | 40  | 60  | 80  | 100 |
|---------------------|-----|-----|-----|-----|-----|
| Total phenolic (mg GAE/100 g) | 51.38±0.03^c | 54.04±0.00^b | 55.17±0.02^ab | 56.83±0.03^a | 56.90±0.01^a |
| Total flavonoid (mg GE/100 g) | 10.27±0.01^c | 13.54±0.00^b | 13.91±0.01^ab | 14.23±0.00^a | 14.26±0.02^a |
| Anthocyanin (mg/100 g) | 18.53±0.02^c | 20.97±0.01^b | 21.29±0.00^ab | 21.86±0.02^a | 21.90±0.03^a |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Table 2. Effect of microwave frequency (GHz) to the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx

| Microwave frequency (GHz) | 10  | 20  | 30  | 40  | 50  |
|--------------------------|-----|-----|-----|-----|-----|
| Total phenolic (mg GAE/100 g) | 56.83±0.03^c | 57.41±0.00^b | 58.63±0.03^a | 59.52±0.02^a | 59.59±0.03^a |
| Total flavonoid (mg GE/100 g) | 14.23±0.00^b | 14.45±0.01^ab | 14.68±0.00^ab | 14.87±0.01^a | 14.90±0.01^a |
| Anthocyanin (mg/100 g) | 21.86±0.02^b | 22.14±0.01^ab | 22.59±0.02^ab | 22.84±0.03^a | 22.87±0.00^a |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)
3. Effect of the Number of Extraction Cycles in Phytochemical Extraction of Hibiscus sabdariffa L. Calyx

The outcome of stirring is related to the mass transfer manner in the solvent phase. Therefore, balance between the aqueous and vapor phases can be achieved more rapidly. The use of agitation in MAE quickens the extraction by enhancing desorption and dissolution of active compounds bound to the sample matrix. Through stirring, the disadvantages of the use of low solvent-to-solid ratio can be reduced, together

Table 3. Effect of microwave irradiation duration (min) to the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx

| Microwave irradiation duration (min) | 5      | 10     | 15     | 20     | 25     |
|-------------------------------------|--------|--------|--------|--------|--------|
| Total phenolic (mg GAE/100 g)       | 59.52±0.02 
|                                    | 64.29±0.00 | 67.15±0.03 | 66.49±0.02 | 62.37±0.00 |
| Total flavonoid (mg GE/100 g)       | 14.87±0.01 
|                                    | 17.75±0.03 | 18.62±0.02 | 18.25±0.01 | 17.13±0.02 |
| Anthocyanin (mg/100 g)              | 22.84±0.03 
|                                    | 23.27±0.01 | 24.01±0.00 | 23.81±0.01 | 23.54±0.00 |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)*

Table 4. Effect of liquid to solid ratio to the total phenolic, flavonoid and anthocyanin extracted from Hibiscus sabdariffa L. calyx

| Liquid to solid ratio | 2:1     | 3:1     | 4:1     | 5:1     | 6:1     |
|-----------------------|---------|---------|---------|---------|---------|
| Total phenolic (mg GAE/100 g) | 67.15±0.03 | 57.25±0.00 | 50.49±0.03 | 29.42±0.00 | 11.63±0.02 |
| Total flavonoid (mg GE/100 g)   | 18.62±0.02 | 14.34±0.03 | 12.18±0.01 | 8.33±0.03  | 5.47±0.01  |
| Anthocyanin (mg/100 g)          | 24.01±0.00 | 21.84±0.01 | 18.53±0.02 | 16.72±0.01 | 14.85±0.03 |

*Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)*

3.5 Effect of Extraction Temperature (°C) in Phytochemical Extraction of Hibiscus sabdariffa L. Calyx

High-temperature extraction can be gainful with the resulting increase in solubility. This is because higher temperature causes increased intermolecular interactions within the solvent, giving increase to higher molecular motion which raises the solubility. The accelerating temperature may cause a cellular pressure build up which may create cell rupture and opening of the cell matrix, and as a result, increased accessibility to be extracted into the solution. When the temperature of water is raised, there is a steady decrease in its permittivity, viscosity and surface tension but an increase in its diffusivity characteristics. The increased temperature can overcome the solute–matrix interaction caused by van der Waals forces, hydrogen bonding, dipole attraction of the solutes molecules and active sites in the matrix [75]. However, thermally labile compounds are degraded at elevated temperatures. Sufficient temperature is necessary to optimize the extraction efficiency, avoid thermal degradation of the target analytes, and to supply reproducible processing conditions [48,53]. Raw Hibiscus sabdariffa L. calyx was extracted by different extraction temperature values (50, 55, 60, 65, 70°C). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were important indicators so they were chosen to identify the optimal extraction temperature. Our result showed that accelerated temperature from 50°C to 70°C can hampered the phytochemicals. There was not significant difference at extraction temperature 50°C and 55°C. Therefore the optimal extraction temperature should be 55°C to receive the highest content of total phenolic, flavonoid and anthocyanin to save duration of extraction. The optimum conditions for fresh and dried roselle calyces, the extraction temperature of 50°C for 30 min [62]. Dried roselle calyces at a ratio of 1:10 (dried roselle calyces: water) were extracted at 50°C for 30 minutes [32].

3.6 Effect of the Number of Extraction Cycles in Phytochemical Extraction of Hibiscus sabdariffa L. Calyx

The outcome of stirring is related to the mass transfer manner in the solvent phase. Therefore, balance between the aqueous and vapor phases can be achieved more rapidly. The use of agitation in MAE quickens the extraction by enhancing desorption and dissolution of active compounds bound to the sample matrix. Through stirring, the disadvantages of the use of low solvent-to-solid ratio can be reduced, together
with the minimization of the mass transfer barrier created by the concentrated solute in a localized region resulting from insufficient solvent. It is possible to observe the difference between suspensions with and without stirring [76]. The microwave cycle used must be carefully monitored, because microwave-assisted extraction offers quick release of the target components in the surrounding extraction solvent and longer extraction durations could accelerate the decomposition of extracted phenolics for extended extraction under these harsh conditions [77]. In our research, raw *Hibiscus sabdariffa* L. calyx was extracted by different numbers of extraction cycles (1, 2, 3, 4, 5). Total phenolic (mg GAE/100 g), total flavonoid (mg GE/100 g), total anthocyanin (mg/100 g) were important indicators so they were chosen to identify the optimal number of extraction cycles. Our result showed that when the number of extraction cycles increased from 1 to 4 cycles, the phytochemical stability increased respectively. However there was not significant difference if we prolonged the extraction cycle over 4 units. Therefore the optimal number of extraction cycles should be four to receive the highest content of total phenolic, flavonoid and anthocyanin.

### 4. CONCLUSION

Roselle (*Hibiscus sabdariffa*) is a good source of phytochemical constituents such as dietary antioxidant, phenolic, ascorbic acid, carotenoid providing high antioxidant activity with potential health benefits. The application of microwave extraction of functional constituents from roselle calyx results in effective manner. Electromagnetic waves are indeed absorbed selectively by media possessing a high dielectric constant resulting in more effective heating. In this research, we have successfully identified major variables influencing to the phytochemical extraction inside roselle calyx under microwave.

### CONSENT AND ETHICAL APPROVAL

It is not applicable.

### COMPETING INTERESTS

Author has declared that no competing interests exist.

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